ARZ01-13997

Ed Kordoski <Kordoski@SOCMA.com> on 10/02/2002 10:31:03 AM

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To: NCIC OPPT/DC/USEPA/US@EPA, Rtk Chem/DC/USEPA/US@EPA
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Subject: Robust Summaries and Test Plan for Nitroglycerin (CAS # 55-63-0) - Registration #

U.S. NPC
Consortium

U.S.Nitroglycerin Producers

[IM
AG
E]

1850 M Street, NW, Suite700, Washington, DC 20036

(202) 721-4145 – (202) 296-8120 fax

E-mail: *kordoskie@socma.com*

September 25, 2002

***Via Certified Mail*/RETURN RECEIPT**

Christine Todd Whitman, Administrator
U.S.Environmental Protection Agency
Post Office Box 1473
Merrifield, VA22116

Attn: Chemical Right-to-Know Program; HPV Challenge Program; HPV Reference Number:

The Synthetic Organic Chemical Manufacturers Association's (SOCMA) U.S. Nitroglycerin Producers Consortium (USNPC) comprised of Alliant Techsystems Inc., Copperhead Chemical

Company Inc., Dyno Nobel Inc., and St. Marks Powder, Inc; as per our commitment to the EPA High Production Volume (HPV) Challenge Program, is submitting our robust summaries and test plan for 1,2,3-trinitroglycerin (TNG) commonly called nitroglycerin (CAS # 55-63-0). Although we committed to submitting this document in 2003, it was completed earlier and is being submitted now both in paper form and on CD.

The final HPV document is comprised of a summary matrix of the required SIDS data points indicating the necessary test, test species, protocol used, and the results; and forty-one individual Robust Summaries. Examination of the SIDS matrix shows data for all the required SIDS tests except for an Inhalation LD_{50}. Undiluted, nearly pure nitroglycerin is an extremely 'sensitive' high explosive and the danger of a major explosion of TNG vapors or mist in an inhalation study, with resulting catastrophic damage to personnel and facility is too great to consider undertaking such a dangerous toxicological study.

Based on the complete matrix of SIDS data elements, except for the missing and extremely dangerous Inhalation LD_{50}, and the results of the biological and toxicological studies along with a very consistent pattern of structural and physicochemical properties no additional testing is required or planned.

Please contact me at (202) 721-4145 if there are any questions relating to this submission.

Sincerely,

Edward W. Kordoski, MBA, Ph.D.

Executive Director, U.S.Nitroglycerin Producers Consortium

cc: USNPC Group Members

Edward W. Kordoski, MBA, Ph.D.

Synthetic Organic Chemical Manufacturers Association, Inc. (SOCMA)

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[IMAGE]

- SIDS_TNG_StabilityAquaticFinal.DOC
- SIDS_TNG_TeratoRatDietaryFinal.DOC
- SIDS_TNG_VapourPressureFinal.DOC
- SIDS_TNG_WaterSolubilFinal.DOC
- SOCMA_TNG_LABELS.doc
- SIDS_TNG_3GenReproRatDietFinal.DOC
- SIDS_TNG_5dayMetHbDogOralFinal.DOC
- SIDS_TNG_Ames1975DMSOFinal.DOC
- SIDS_TNG_Ames1980DMSOFinal.DOC
- SIDS_TNG_Ames1986EthanolFinal.DOC
- SIDS_TNG_BiodegCsourceFinal.DOC
- SIDS_TNG_BiodegSelfInitFinal.DOC

- SIDS_TNG_BoilingPointFinal.DOC
- SIDS_TNG_CHO_MutFinal.DOC
- SIDS_TNG_ChromAbsKidDogSubCapsulFinal.DOC
- SIDS_TNG_ChromAbsKidRatSubOralFinal.DOC
- SIDS_TNG_ChromAbsLymphDogSubCapsulFinal.DOC
- SIDS_TNG_ChromAbsLymphRatSubDietFinal.DOC
- SIDS_TNG_ChromAbsRatKidChronDietFinal.DOC
- SIDS_TNG_ChromAbsRatMarrowChronDietFinal.DOC
- SIDS_TNG_DomLethalRatSubDietFinal.DOC
- SIDS_TNG_EC50AlgaAcuteFinal.DOC
- SIDS_TNG_FugacityFinal.DOC
- SIDS_TNG_InhalFinal.DOC
- SIDS_TNG_IrritRabbAcuteEyeFinal.DOC
- SIDS_TNG_IrritSkinRabbit.DOC
- SIDS_TNG_LC50DaphniaAcuteFinal.DOC
- SIDS_TNG_LD50MinnowAcuteFinal.DOC
- SIDS_TNG_LD50MouseAcuteOralFinal.DOC
- SIDS_TNG_LD50RatAcuteDermFinal.DOC
- SIDS_TNG_LD50RatAcuteOralFinal.DOC
- SIDS_TNG_LD50TroutAcuteFinal.DOC
- SIDS_TNG_MLDRabbIV_Final.DOC
- SIDS_TNG_MPtFinal.DOC
- SIDS_TNG_OralDog1yrCapsulFinal.DOC
- SIDS_TNG_OralMouseChronDietFinal.DOC

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SIDS ROBUST SUMMARIES
FOR 1,2,3-TRINITROGLYCERIN
(CASRN: 55-63-0)

TWO FOLDERS
- TNG FINAL SUMMARIES (41)
- TNG RECAP TEST RESULTS (1)

SYNTHETIC ORGANIC CHEMICALS MFG. ASSN.
(SOCMA)

SIDS ROBUST SUMMARIES
FOR 1,2,3-TRINITROGLYCERIN
(CASRN: 55-63-0)

TWO FOLDERS
- TNG FINAL SUMMARIES (41)
- TNG RECAP TEST RESULTS (1)

SYNTHETIC ORGANIC CHEMICALS MFG. ASSN.
(SOCMA)

SOCMA_TNG_LABELS

1,2,3, -TRINITROGLYCERIN (TNG)
CAS RN: 55-63-0
SUMMARY OF SIDS RESULTS

SIDS SEGMENT / TEST		SPECIES	PROTOCOL	RESULTS
PHYSICAL-CHEMICAL				
1	Melting Point (°C)	--	--	2.3 ; 13.0
2	Boiling Point (°C)	--	--	Inert atmosphere： ~ 240 (calc'd) Air: Decomposes = 70
3	Vapor Pressure (kPa)	--	--	20°C = 3.6 x 10^{-5}; 40°C = 3.3 x 10^{-4}; 60 °C = 2.3 x 10^{-3} (Best represent. values)
4	Partition Coefficient	--	--	1.62 – 2.35
5	Water Solubility (g/L)	--	--	20°C = 1.38 – 2.0; 40°C = 1.68 60°C = 1.96 – 2.5; 80°C = 3.44
ENVIRONMENTAL				
6	Photodegradation ($t_{1/2}$; days)	--	Footnote 1	Distilled water = 116 Municipal pond H_20= 57 H_2O downriver from plant = 73
7	Water Stability ($t_{1/2}$, days)	--	--	pH 9 @ 25 °C = 37; pH 5 @ 25 °C = 8575[2] ; pH3 @ 37°C = ">3650" .
8	Fugacity	--	EQC software	Predominantly water & soil[3].
9	Biodegradation	Indigenous microorganisms downriver from plant	Footnote 1	1st Order rate const 0.6/hr. – 8.6 x 10^{-2}/hr 2nd Order constant 1.06 – 1.43 x 10^{-9} ml/cell/hr
9	Biodegradation	Sewage microorganisms	Footnote 1	90-100 % metabolism to DNG & MNG
ECOTOXICOLOGY				
10	96 hr. LC_{50}	Rainbow trout	ASTM E729-80	1.90 mg / L
10	"	F'head Minnow	"	3.58 mg / L
10A	8 day dietary LD_{50}	Quail *c. virginianus*	Same as OECD No. 205	>5620 ppm; LOAEL = 5620 ppm; NOAEL = 3160 ppm.
11	96 hr. EC_{50}	Alga **S.capricornutum**	EPA 797.1060	1.15 mg / L
12	48 hr. LC_{50}	Daph. *C. dubia*	ASTM E729-80	17.83 mg / L
HEALTH				
13	Acute oral LD_{50}	Mouse	Footnote 1	M: 1,188 mg / kg (95% C.L.= 1008-1352). F: 1055 mg / kg (95% C.L. = 895 - 1178)
13	Acute oral LD_{50}	Rat	16 CFR 1500.3 (c)(2)(I) [FHSA]	685 (95% CL=510 – 940) mg / kg
13	I.V. MLD	Rabbit	Footnote 1	45 mg / kg
13	Dermal LD_{50}	Rabbit	Footnote 1	>9,560 mg / kg
13	Inhalation LD_{50}	--	--	Not performed. Too dangerous
13	Skin irritation	Rabbit	16CFR1500.41 (FHSA)	Primary Irritation Index = 0.90

13	Eye irritation	Rabbit	16CFR 1500.42 (FHSA)	Very mild conjunctival irritation
13	Skin sensitization	Guinea pig	Kligman Maximization	4 / 10 sensitized
13A	Skin sensitization	Humans	Clinical study	Of ~6200 cases occupat'l dermatitis seen at Finnish gov't. OSH clinic, only 4 were confirmed due to TNG
GENETIC TOXICITY *IN VIVO*				
14	Dominant Lethal (diet)	Rat	~Same as OECD #478	No effect at maximum test level. (1.0 % in diet for 13 weeks)
14	Chromosome aber-rations (diet)	M & F Rats (kidney cells)	~Same as OECD #475	No apparent effect @ ~230 mg/kg for eight weeks.
14	Chromosome aber-rations (diet)	M & F Rats (lymphocytes)	~Same as OECD #475	No apparent effect @ ~230 mg/kg for eight weeks.
14	Chromosome aber-rations (diet)	M & F Rats (bone marrow)	~Same as OECD # 475	No apparent effect @ 360 mg/kg (M) or 430 mg/kg (F) for 2 years.
14	Chromosome aber-rations (diet)	M & F Rats (kidney cells)	~Same as OECD # 475	No apparent effect @ 360 mg/kg (M) or 430 mg/kg (F) for 2 years.
14	Chromosome aber-rations (oral capsule)	M & F Dogs (kidney cells)	~Same as OECD # 475	No apparent effect @ 5 mg/kg for eight weeks.
14	Chromosome aber-rations (oral capsule)	M & F Dogs (lymphocytes)	~Same as OECD # 475	No apparent effect @ 5 mg/kg for eight weeks.
GENETIC TOXICITY *IN VITRO*				
15	CHO-K1 cells	Chinese hamster (K-1 cells)	~Same as OECD #476	No mutagenic effects.
15	Ames (TNG in DMSO; 1980)	S. typhimurium mutants	~Same as OECD #471	Active in TA 1537 @ 1mg a.i. / plate.
15	Ames (TNG in Ethanol)	S. typhimurium mutants	~Same as OECD #471	Active in TA 1535 @ 0.5 & 1.5 mg / plate .
15	Ames (TNG ad-sorbed on lactose. Suspended in DMSO; 1975)	S. typhimurium mutants	~Same as OECD #471	Active in TA 1535 @ 0.75 & 1.0 mg / plate
REPEATED DOSE TOXICITY				
16	1 Yr. Oral Capsule	M & F Beagle dog	~Same as OECD #452	Methemoglobin formation in M & F at lowest dose (1 mg / kg).
16	2 Yr. Diet	M & F Rat	~Same as OECD #452	Increased foci of hepatocellular alteration (both sexes). Spleen hyperpigmentation Interstitial cell tumors (male testes). Kidney epithelial

				hyperpigment'n Multiple blood chem. changes Incr. liver neoplastic nodules and Hepatocellular carcinomas NOAEL: M = 3.04 mg TNG/kg/day F = 3.99 mg/kg TNG/day TD_{50}[5] M, mixed liver = 221 mg/kg/day M, Testic. interst.=405 mg/kg/day F, mixed liver = 329 mg/kg/day
16	2 Yr. Diet	M & F Mouse	~Same as OECD #452	Decr. food intake & wt. gain; behavioral effects; metHb format'n. NOAEL: M = 114.6 mg / kg /day F = 96.4 mg / kg / day
17	3-Gen. Reproduction	Rat	U.S. FDA (1966)	Aspermatogenesis in F2a males[4]. Decr. food intake in F_{1b} Females[4]. Reduced total litter wt. (F_{1b})[4]. NOAEL: M = 39 mg/kg/day F = 46 mg / kg / day
18	Teratogenicity	Rat	U.S. FDA (1966)	Decr. b.wt., high dose. Incr. diaphrag. hernias, high dose Decr. hyoid bone ossific, high dose
SPECIAL	Methemoglobinemia protection / antidote	M & F Beagle dog	Footnote 1	Methylene blue depresses MetHb formation; does not hasten removal.

1. Investigator's procedure. See Robust Summary.
2. Calculation by Robust Summary author, based on $t_{1/2}$ = 134 days @ 85°C. Assumes reaction rate halves for every 10°C drop in temperature.
3. Level III values in water and soil lower than Level II values. Amounts of decrease vary depending on effects of water pH, photolysis, and biodegradation.
4. At 1% dietary dose.
5. TD_{50} (50% tumor dose); see Gold & Zeiger (1997).

AR201-13997B

PHYSICAL / CHEMICAL ELEMENTS
1) MELTING POINT

2002 OCT 18 AM 11:15

TEST SUBSTANCE

- Nitroglycerin (TNG, 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trade names and other trivial names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM data bases. The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 901002-0 is fisted as "SNG" (no further identification) in this Handbook section. Number 80066-48-4 is listed in this Handbook section as 1,2,3-propanetriol,1,2,3-trinitrate, the systematic name for TNG.

METHOD

Except the information from Kemp, *et* al.(1957), all melting points were obtained from secondary sources which did not give details of the investigator's methods.

METHOD FOLLOWED: Kemp, *et al.* (1957), measured temperatures with a No. 36 gage copper-constantan thermocouple in conjunction with an L & N K-2 potentiometer and a high sensitivity mirror-type galvanometer., The thermocouple was calibrated from a deviation curve for the region above 0°C by determining the e.m.f values at the f.pt. of mercury (-38.87°C) and the normal sublimation point of $C0_2$ (78.51°C), and drawing a line through the points and the origin (0°C). They claimed an accuracy of ±2uv for this method. For the region above 0°T the thermocouple was calibrated at the b.pt. of water and the m. pts. of methyl naphthalene (33.62°C) and naphthalene (79.810°C). The purity of these latter two materials previously had been established by m.pt. measurements using a platinum resistance thermometer.

GLP: All studies cited probably did not follow GLP guidance, although good scientific procedures probably were used.

YEAR Year results were obtained was never given in any publication. Year of publication is shown in the table below.

RESULTS

TNG has been reported to crystallize (freeze) into two isomeric crystalline forms, with different freezing and melting points, as shown and referenced in the Table below. The crystalline isomer freezing at ~2.0°C has been reported to crystallize in a triclinic habit (Hibbert, 1912), while the one freezing at ~13°C has been reported to crystallize in a rhombic habit (Hibbert, 1912; Urbanski, 1965). Pictures of the two forms are shown in Hibbert (1912) and in Urbanski (1965).

Freezing Points (°C)		Melting Points (°C)			
Isomer 1[a]	Isomer 2[a]	Isomer 1[a]	Isomer 2[a]	Reference	Year Publ'n.
2.2	13.2	2.8	13.5	Kast	1908
1.9	13.0	2.0	13.2	Hibbert	1912
1.9	13.0	n.d.[b]	n.d.	Hackel	1936
n.d.[b]	n.d.	2.2	12.2	Will	1908
n.d.	13.3	n.d.	n.d.	Nauckhoff	1911
n.d.	12.83[C]	n.d.	n.d.	Kemp	1957
n.d.	12.86[d]	n.d.	n.d.	Kemp	1957

a. Isomer 1 has a triclinic structure and Isomer 2 has a rhomboid structure.
b. Not determined.
c. Test material prepared by mixed acid (sulfuric + nitric) nitration
d. Test material prepared by nitration with nitric acid only.

CONCLUSIONS & DATA QUALITY

Considering that the results reported in the table above were obtained in six different laboratories, over a span of ~50 years, and in laboratories in both Europe and the U.S., the concordance between the various investigators is very good. Normally, one would assume that the sample with the lowest freezing temperature and the highest melting temperature is the purest and gives the truest numbers. However, the rate of heating and of cooling can affect the results of both of these measurements. Since none of these authors published this information, the author of this Robust Summary suggests that the Best Representative Value (BRV) is the average of all the values in each category. Accordingly, the BRVs for the **freezing points** for the two crystalline isomers are **2.0°C** and **13.0°C**, respectively, and the BRVs for the **melting points** for the two isomers are **2.3°C** and **13.0°C**, respectively.

REFERENCES

Canadian **C**enter for **O**ccupational **H**ealth and **S**afety (**2001**). CHEMINDEX CDROM Issue 2001-4.

Kemp, M. D., S. Goldhagen, and F.A. Zahlman (**1957**). *J Phys. Chem.,* 61, 240-42.

Hibbert, H. (**1912**). The preparation, crystallin (sic) structure and physical properties of the two forms of solid nitroglycerin. *Orig. Comm. 8th Internat. Congr. Appl. Chem.,* **4**, 37-57; from *Chem. Abstr.* (1912), **6**(19) 3523.

Urbanski, T. (**1965**). Chemistry and Technology of Explosives, Vol. II, Pergammon Press, New York; pp. 32-61.

Kast, H. (**1908**). On the freezing and melting point of nitroglycerol. *Z ges. Schiess. Sprengstoffw.* 1, 2009; from *Chem. Abstr.* **2**(9), 1346.

Hackel, J. (**1936**). Investigation of the properties of the two modifications of nitroglycerin. *Roczniki Chem.,* **16**, 213-22; from *Chem. Abstr.* (1936), 30, 8619.

Will, W. (**1908**). *Berichte* (May 9),1107-125; from *Chem. Abstr.* **2**(9), 2016.

Nauckhoff, S. (**1911**). The crystallographic properties of nitroglycerin. *Z ges. Schiess. Sprengstoffw.* **6**, 124-5; from *Chem. Abstr.* **5**(9), 2331.

PHYSICAL / CHEMICAL ELEMENTS
2) BOILING POINT

TEST SUBSTANCE

1. Nitroglycerin (TNG;1,2,3-propanetriol, 1,2,3-trinitrate; CASRN: 55-63-0).

2. Synonyms include almost 100 trade names and other trivial names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed in the National Library of Medicine (NLM) "ChemIDplus" data base for TNG. These are all cross-referenced to 55-63-0 in the NLM and Canadian Center for Occupational Health and Safety data bases. The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is listed as "SNG" (no further identification) in this Handbook section. Number 80066-48-4 is listed in this Handbook section as 1,2,3-propanetriol,1,2,3-trinitrate, the systematic name for TNG.

METHOD

Belyaev and Yuzefovich (1940) calculated a boiling point for TNG in anoxic/low oxygen atmospheres based on their experimentally determined B.Pts. of NG at 2 mmHg (0.026 kPa), and 50 mmHG (6.7 kPa), but the methodology used to measure vapor pressures was not reported in the secondary source available to the author of this Robust Summary. The author of this Robust Summary determined the B.Pt. of TNG in low oxygen/anoxic conditions by extrapolating the vapor pressure line from the 0.46 mmHg (0.061 kPa; 100°C) maximum used in the VAPOR PRESSURE SIDS document to 760 mmHg on vapor pressure graph paper.

The dates of all the primary references (1904 – 1940) suggest that strict GLP procedures were not followed. However, physical scientists of the eras in which these determinations were made, usually did very competent work, and the consistency of the data used in both of the SIDS documents, suggests strongly that the work is reliable.

RESULTS

Belayef & Yuzefovich(1940) report a B.Pt. of 125°C at 2 mmHg (0.27 kPa) pressure, and a boiling point of 180°C at 50 mmHg (6.7 kPa)[2] pressure for TNG. These same authors reported a calculated B.Pt. of 245 ± 5°C based on these two experimentally determined B.Pts. under anoxic/low oxygen conditions The extended "Best Representative Line" through the data points used in the Vapor Pressure SIDS document, as mentioned above, gave an expected B.Pt. of 238°C ± "a few degrees" at atmospheric pressure in the absence of oxygen, or in the presence of only small amounts of oxygen.

DISCUSSION and CONCLUSIONS

At typical levels of oxygen in the atmosphere, TNG has been reported to: 1) be stable at 50°C (Brandner, 1938) 2) begin to decompose after heating for a few hours at 70°C (Marshall, 1904), 3) decompose rapidly at temperatures of 90°C and above (Marshall & Peace, 1916), 4) evolve "nitrous yellow" vapors at 135°C(Budavari, 1996), 5) sublime rapidly at 160°C(Will, 1908), 6) explode at 218°C (Lide, 1995,1996), and 7) boil and/or explode at 260°C (USEPA ACQUIRE Data Base,1993; Hazardous Substances Data Bank, 2000; National

Library of Medicine TOXNET Data Base, 2002; Verschueren, 1996). References 9, 10, 11, and 12 appear to have obtained their information from the same source, but none cited a primary reference.

The boiling point of TNG at atmospheric pressure is not determinable because of this explosivity and decomposition at elevated, but below boiling, temperatures in the presence of oxygen. However, even at 180°C, no evidence of decomposition was reported by Belyaev & Yuzefovich (1940) when the vessel pressure was =50 mmHg (6.7 kPa). The author of this SIDS document believes that the evidence in the VAPOR PRESSURE Robust Summary in this set of Robust Summaries and this Robust Summary indicates that the B.Pt. of TNG in anoxic/low oxygen conditions is 240°C ± a "few degrees".

DATA QUALITY

The author of this Robust Summary believes the data in the primary references cited are reliable.

REFERENCES

Canadian **C**enter for **O**ccupational **Health** and **S**afety (**2001**), *CHEMINDEX CDROM* Issue 2001-4.

Belyaev, A. F. & N. A. **Yuzefovich** (**1940**), *Compt. Rend. acad. Sci., U.R.S.S.* **27**, 133-35.

Brandner, J. D., (**1938**), *Ind. Eng. Chem*. **30**, 681-84.

Marshall, A. (**1904**), *J. Soc. Chem. Industry* **23**, 154-58.

Marshall, A. and G. **Peace** (1916), *J. Chem. Soc*. **109**, 298-302.

Budavari, S. Ed. (**1996**), The Merck Index, Merck & Company, Inc., Whitehouse Station, NJ.

Will**, W. (**1908**), *Berichte* May 9, 1107-25.**

Lide, D. R. (**1995**,**1996**), Handbook of Chemistry and Physics, CRC Press, Boca Raton, p. **3**-279.

U.S.E.P.A. (**1993**), ACQUIRE Data Base; from CCOHS CESAR data base.

Hazardous Substances Data Bank **(**2000**).**

National Library of Medicine **(**2002**), TOXNET.**

Verschueren, K. (1996), Handbook of Environmental Data on Organic Chemicals, van Nostrand Reinhold, New York, NY, p.1392.

SIDS_TNG_BoilingPointFinal

PHYSICAL / CHEMICAL ELEMENTS

3) VAPOUR PRESSURE

TEST SUBSTANCE

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for (TNG) in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occu-pational Health & Safety (CCOHS) toxicological data bases. The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propane-triol,1,2,3-trinitrate, the systematic name for TNG.

INTRODUCTION

Many values for the vapor pressure of TNG at various temperatures have been published in the chemical literature from 1904 – 1966. Study temperatures have varied from 20°C to 180°C. No one investigator or team of investigators studied the entire range, and various methodologies were used. There has been controversy over the best methodology, and no consensus was ever reached. Consequently, the author of this Robust Summary felt the best approach was to graph all the published data on vapor pressure (log p *vs*. 1 / T) graph paper and see if any order or regularity was apparent. Any such order that appeared between investigators would be lost if a separate Robust Summary was prepared for each publication. Therefore, this author decided to review all of the publications in one Robust Summary and present his conclusions about the best representative values. The data cover not only the above-mentioned temperature range, they also cover almost six orders of magnitude of vapor pressures. All of the original data are presented in this Robust Summary, and any reader is free to analyze it in any other way.

METHODS

Nine publications were found in the chemical literature that reported values for the vapor pressure of nitroglycerine at various temperatures. As discussed below, after detailed analysis of the information from these papers, six of them were found to be consistent with each other. The six were from four different investigators or groups of investigators, and one was from a generally recognized reference compendium that did not give original references (Lide and Frederikse, 1996).

Basically, two methods were used by the authors of the ten papers cited, to determine the most likely vapor pressures of TNG. One of the methods was a "static" method, *e.g*., Naoum & Meyer (1929). The second was a "dynamic" method, *e.g*., Kemp, *et al*. (1957). The method(s) used by each investigator are given in the Table in the **RESULTS** Section.

In the static method, TNG was added to a suitable flask. The flask was then flushed with "pure, dry" air, closed, and weighed. The flask and contents were then equilibrated at the desired temperature for a period of time. A sample from the headspace was then withdrawn and analyzed for TNG or, alternatively, the headspace was quickly flushed and the flask and contents reweighed. In the dynamic method, a measured volume of "pure, dry" air at sample temperature was slowly bubbled through the sample of TNG, which was in a series of Geissler bulbs, or their equivalent, in a constant temperature bath. The bulbs, with their samples of TNG, were weighed before placing in the bath. After the measured volume of air had passed through the TNG in the bulbs, the bulbs with contents were removed from the bath, cleaned, dried, and reweighed. This latter procedure was validated by one set of investigators by determining the vapor pressure of freshly boiled distilled water before the first TNG experiment.

In the Brandner (1938) modification of the Crater method (1929), an additional drying step for the TNG was used prior to adding it to the Geissler bulbs. Marshall & Peace (1916) used ground, dried, guncotton (30% TNG) instead of liquid TNG and slowly passed the measured volume of dried air through a **U** tube packed with the sample and immersed in the constant temperature bath. They trapped the entrained TNG in a cold trap and weighed the condensate instead of weighing the Geissler bulbs before and after. The Kemp (1957) group, used the Brandner modification of the Crater Method.

Belyaev and Yuzefovich (1940) determined b.pts at 2 mm and 50 mm pressure. Since the b.pt. is the temperature at which a chemical's vapor pressure equals the atmospheric pressure, this work provided an indirect way of determining vapor pressures. Since oxygen levels would be low under these conditions, the method had the advantage of providing vapor pressures under conditions where oxidative changes were minimal. The method used by them was not reported in the abstract from which their data were obtained.

Three data points were obtained from the CRC Handbook of Chemistry & Physics (Lide and Frederikse, 1996). The source(s?) of the data was (were) not given. One of the three points is very close to one from Marshall and Peace (1916), and could be from their study, except the latter didn't report any studies at the other two points given in the Handbook. These other points are consistent with those of other scientists who made vapor pressure measurements at or near those temperatures, as discussed in the **DISCUSSION** Section.

The original studies were published during the years 1904 – 1966. The work was done in a total of eight different laboratories in Europe, Japan, and the U.S. The laboratories probably did not adhere to the letter of GLP guidance (predated it by at least 12 years), although they may have more-or-less followed the spirit of GLP. However, none of the publications gave that degree of detail. The consistency of the results from four laboratories (U.S. and Europe; 1904-1966), over almost six orders of magnitude of vapor pressure and from 20°C to 180°C, argues for the reliability of their data (Comment of author of Robust Summary).

RESULTS

The vapor pressure values for TNG from 20 – 180°C, inclusive, as determined in the presence of oxygen by the various investigators, are shown in the table below.

REPORTED VAPOUR PRESSURES FOR NITROGLYCERIN

	VAPOR PRESSURE					
TEMP. (°C)	MmHg	Kpa	No. REPS.	METHOD	REFERENCE	YEAR
20	2.0×10^{-4}	2.66×10^{-5}	2	Dynamic a	Kemp (Sample II)	1957
	2.1×10^{-4}	2.79×10^{-5}	3	Dynamic a	Kemp (Sample I)	1957
	2.5×10^{-4}	3.33×10^{-5}	2	Dynamic	Marshall & Peace	1916
	1.5×10^{-3}	2.0×10^{-4}	Unknown	Unknown	Rinkenbach	1965
	9×10^{-3}	1.2×10^{-3}	1	Static	Naoum & Meyer	1929
	1.1×10^{-2}	1.47×10^{-4}	1	Dynamic	Naoum & Meyer	1929
25	1.8×10^{-3}	2.4×10^{-4}	2	Dynamic	Crater	1929
30	3.8×10^{-4}	5.07×10^{-5}	1	Dynamic	Marshall & Peace	1916
	8.2×10^{-4}	1.09×10^{-4}	3	Dynamic	Kemp (Sample I)	1957
	1.1×10^{-3}	1.47×10^{-4}	3	Dynamic	Kemp (Sample II)	1957
	1.2×10^{-3}	1.6×10^{-4}	2	Dynamic	Brandner (Sample A) b	1938
35	2.0×10^{-3}	2.7×10^{-4}	1	Dynamic	Brandner (Sample B) b	1938
	4.6×10^{-3}	6.1×10^{-4}	2	Dynamic	Crater	1929
	3.6×10^{-2}	4.8×10^{-3}	1	Dynamic	Naoum & Meyer	1929
40	2.4×10^{-3}	3.2×10^{-4}	2	Dynamic	Marshall & Peace	1916
	3.0×10^{-3}	4.0×10^{-4}	2	Dynamic	Brandner (Sample A)	1938
	3.2×10^{-3}	4.3×10^{-4}	3	Dynamic	Kemp (Sample I)	1957
	3.6×10^{-3}	4.8×10^{-4}	3	Dynamic	Kemp (Sample II)	1957
45	4.7×10^{-3}	6.3×10^{-4}	1	Dynamic	Brandner (Sample B)	1938
	1.3×10^{-2}	1.72×10^{-3}	2	Dynamic	Crater	1929
50	7.2×10^{-3}	9.6×10^{-3}	2	Dynamic	Marshall & Peace	1916
	7.5×10^{-3}	1×10^{-3}	Unknown	Unknown	Lide & Frederikse	1996
	8.1×10^{-3}	1.8×10^{-3}	2	Dynamic	Brandner (Sample A)	1938
55	3.6×10^{-2}	4.77×10^{-3}	2	Dynamic	Crater	1929
60	1.8×10^{-2}	2.4×10^{-3}	1	Dynamic	Marshall & Peace	1916

	6.0×10^{-2}	8.0×10^{-3}	Unknown	Dynamic	Rinkenbach	1965
70	4.3×10^{-2}	5.7×10^{-3}	2	Dynamic	Marshall & Peace	1916
	5.1×10^{-2}	6.8×10^{-3}	Unknown	Static	Marshall	1904
75	7.5×10^{-2}	1×10^{-2}	Unknown	Unknown	Lide & Frederikse	1996
80	9.8×10^{-2}	1.3×10^{-2}	2	Dynamic	Marshall & Peace	1916
90	2.3×10^{-1}		2	Dynamic	Marshall & Peace	1916
93	2.9×10^{-1}	3.9×10^{-2}	2	Dynamic	Marshall & Peace	1916
100	3.2×10^{-2}	4.3×10^{-2}	Unknown	Unknown	Lide & Frederikse	1996
125	2.0	2.7×10^{-1}	Unknown	Unknown	Belyaev & Yuzefovich[c]	1940
180	50	6.7	Unknown	Unknown	Belyaev & Yuzefovich	1940

1. Sample I prepared by nitration with mixed nitric/sulfuric acids. Sample II prepared by nitration with only nitric acid.
2. Samples A & B were prepared in separate batches from the same reactants and under the same conditions.

c. Determined under anoxic/low oxygen conditions.

In the presence of oxygen, TNG is stable at 50°C (Brandner, 1938), but begins to decompose "very slightly" after ~ 4 hrs. at 70°C (Marshall, 1904), more rapidly at ~90°C (Marshall & Peace, 1916), and vaporizes quickly at 160°C (Marshall, 1929) without melting. See also BOILING POINT SIDS document. In the absence of oxygen, or at low concentrations of oxygen, it appears to be stable (for at least short periods of time) to at least 180°C (Belyaev & Yuzefovich, 1940).

CONCLUSIONS

The best representative values (kPa) for the vapor pressure of TNG in the range 20 - 100°C, in the presence of oxygen, were determined by drawing the best fit line through the data points from references Brandner (1938), Kemp, *et al*. (1957), Lide & Frederikse (1996), Marshall (1904), Marshall & Peace (1916), and Belyaev & Yuzefovich (1940). As discussed in detail in the **DISCUSSION** section, the test samples from references Crater (1929), Naoum & Meyer (1929), and Rinkenbach (1965) probably were contaminated with water. Their data were plotted on the same chart as that of the above six publications, but did not fit the line of the other six publications. The vapor pressure values (**in kPa**) at decade intervals from 20-100°C, determined from this best-fit line, are:

20°C = 3.6×10^{-5} kPa; **30**°C = 1.1×10^{-4}; **40**°C = 3.3×10^{-4}; **50**°C = 8.7×10^{-4}; **60**°C = 2.3×10^{-3}; **70**°C = 5.5×10^{-3}; **80**°C = 1.3×10^{-2}; **90**°C = 2.8×10^{-2}; **100**°C = 6.0×10^{-2}.

When this "Best Fit" aerobic line was extended to 200°C, the anoxic/low oxygen data of Belyaev & Yuzefovich were found to fit it very well. This suggests that the degradation of TNG at = 70°C is probably an oxidative degradation rather than solely a thermal degradation. The anoxic/low oxygen vapor pressures for TNG (**in kPa**) from 110 - 200°C, at decade intervals, are thus expected to be:

110°C = 0.13 kPa; **120**°C = 0.24; **130**°C = 0.45; **140**°C = 0.8; **150**°C = 2.7; **160**°C = 2.7; **170**°C = 4.5; **180**°C = 7.5; **190**°C = 13; **200**°C = 20.

DATA QUALITY

As discussed below, the author of this SIDS document believes that the data from references, Brandner (1938), Kemp, *et al*. (1957), Lide & Frederikse (1996), Marshall (1904), Marshall & Peace (1916), and Belyaev &

Yuzefovich (1940) are consistent, apparently accurate, and suitable for obtaining the vapor pressures reported above in the **CONCLUSIONS** Section. **KEY STUDIES** are flagged in the **REFERENCES** Section.

DISCUSSION

(This discussion reflects the opinion of the author of this SIDS document). Spanggord (1980) compared vapor pressures for TNG at 20°C reported by three investigators [Yokogawa, *et al*. (1966), Kemp, *et al*. (1957), and Vacek and Stanek (1959) and concluded that the data were too disparate to be useful. As shown in the above Table, Kemp, *et al.* (1957), reported average values of 0.20 "µ" [µmHg; see Lide & Frederikse (1996), p. **1**-35] for the vapor pressure of their Sample "b" and 0.21 "µ" (µmHg) for their Sample "a" in the presence of oxygen at 20°C. Since torr ? mmHg, these values convert to 2.0×10^{-4} torr and 2.1×10^{-4} torr, respectively, not the 2.0×10^{-2} torr reported by Spanggord. While Yokogawa still is not in total agreement with Kemp, *et al*., the two now differ by only 1.8×10^{-6} mmHg (2.4×10^{-7} kPa) instead of almost 20×10^{-6} mmHg (24×10^{-7} kPa), and are now part of the same universe. And the over-all picture of nitroglycerin vapor pressure variations with temperature, as shown in the above table, is not as bad as Spanggord implies.

In fact, the concordance of the data from the investigators (with the exception of Naoum & Meyer (1929), Crater (1929), and Rinkenbach (1965) is quite good. If you plot the data for the temperature range from 20 – 100°C obtained from references Brandner (1938), Kemp, *et al*. (1957), Lide & Frederikse (1996), Marshall (1904), Marshall & Peace (1916), Belyaev & Yuzefovich (1940), Naoum & Meyer (1929), Rinkenbach (1965), Vacek and Stanek (1959), and Yokogawa, *et al*. (1966), on vapor pressure graph paper (log p_{10} *vs*. 1/T), the data reasonably well fit three straight, essentially parallel, lines. And the points cover *ca*. four orders of magnitude of TNG vapor pressure The data points of Naoum & Meyer are truly outliers, however, having vapor pressure values *ca*. an order of magnitude greater than those found by most other investigators.

Ignoring the data from Naoum and Meyer (1929), there are 33 data points. There clearly are two, essentially parallel, lines that could be drawn through the data points for this interval. The data points fit their respective best line very well, although the Vacek & Stanek number, as reported by Spanggord (1980), also would be an outlier. However, Vacek & Stanek claimed their data agreed with the values of Marshall (1904), and of Kemp, *et al*. (1957), so this may be another Spanggord error.

The four data points of Crater (1929) and the two data points of Rinkenbach (1965) fit the upper line very well.

The five data points from Brandner (1938), the six from Kemp (1957), the three from Lide & Frederikse (1996), the one from Marshall (1904), the nine from Marshall & Peace (1916), the one from Yokogawa [as reported in Spanggord (1980)], and the two anoxic/low oxygen ones from Belyaev & Yuzefovich (1940) [Total = 26] all fit a second, lower, and essentially parallel, line very well.

Initially, Marshall (1904) thought that the higher values reported by Naoum & Meyer (1929) were due to one or both of two causes: 1) water and other impurities in their sample that were volatilizing, or 2) the dynamic method they used, *vs*. the static method Marshall (1904) was using at that time. However, Naoum & Meyer (1929) also reported high values for the vapor pressure of TNG using a static method (as reported in the Table above) and a high value for the vapor pressure of 1,2-dinitroethylene glycol. In addition, the *Chemical Abstracts* reviewer also pointed out other inconsistencies in the data of Naoum & Meyer (1929). Naoum & Meyer claimed that their samples were carefully purified and then dried "*in vacuo*". It is not reported in the abstract whether they analyzed their samples before carrying out their experiments. Regardless, their results were so divergent from all the other investigators that they must be viewed with suspicion. As discussed below, their samples probably contained larger amounts of water and/or other volatile impurities than did those from the six investigators mentioned above.

Urbanski (1965) felt that the higher values reported by Crater (1929) and by Rinkenbach (1951) might also be due to water contamination in their samples. The drying procedure of Crater was similar to that of Brandner (1938), except Crater did not bubble dry air through the sample for 24 hours as a final drying step, as Brandner did. Kemp, *et al*. (1957), Marshall (1904), and Marshall & Peace(1916) did not describe their drying procedures. Brandner, Kemp *et al*., and Crater did nitrogen analyses on their final, dried, product. Brandner found 18.44 and 18.45%

nitrogen; Kemp, *et al*. found 18.47% nitrogen for Sample 1 and 18.48% nitrogen for Sample II; and Crater found 18.42% nitrogen in his sample. The theoretical value is 18.51%. The value for nitrogen in Crater's sample does not seem seriously below those reported by the other investigators. However, Brandner reported that he found residual water in his samples if he used the drying procedure of Crater and that he therefore initiated a final dry air purge to remove the last traces of water from his samples. He also reported that before initiating this additional drying step, he got higher, and variable, results for his vapor pressure measurements than he did after he added the dry air purge step. In addition, he also reported that he found the pressure in the Geissler bulbs to be below atmospheric pressure, instead of at atmospheric pressure, as Crater had assumed. He claimed that Crater's erroneous assumption also would result in higher values, if the pressure values were not corrected, as he did. On this basis, it seemed reasonable to the author of this SIDS document to conclude that the Rinkenbach (1965) samples also contained residual water and therefore the data points from a) Naoum & Meyer (1929), from b) Crater (1929), and from c) Rinkenbach, could be excluded from the final analysis of the available vapor pressure values for TNG.

Another source of error at temperatures of =70°C is the reported decomposition of TNG at this temperature in the presence of oxygen. With the static method, if the decomposition products are at least as volatile as TNG, they can lead to erroneously high vapor pressure values if they contain nitrogen and the headspace analytical method is nitrogen determination.

With the dynamic method, decomposition of TNG also can lead to erroneous results at temperatures =70°C. Weighing the reservoir and its contents before and after the experiment also can lead to vapor pressure errors on the high side if any of the decomposition products are at least as volatile as TNG. Weighing the condensate in the exhaust air also will give erroneously high values if the temperature of the condensing trap is below the condensation temperature of any decomposition product.

All of the data points from the temperature range 70°C - 100°C fit the Best Representative Line very well, suggesting that these types of errors did not occur in that region during the short time it took to make the measurements [Belyaev & Yusefovich (1940)].

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PHYSICAL/CHEMICAL PROPERTIES
4) PARTITION COEFFICIENT

TEST SUBSTANCE

- Nitroglycerin (1,2,3-propanetriol, 1,2,3-trinitrate; CASRN: 55-63-0)

- Synonyms include almost 100 names (trade names and other trivial names[1]).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for nitroglycerin (TNG) in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is listed as "SNG" (no further identification) in this Handbook section. Number 80066-48-4 is listed in this Handbook section as 1,2,3-propanetriol,1,2,3-trinitrate, the systematic name for TNG.

METHOD

- Methods are not known. This information is from Hazardous Substances Data Bank (2000), and was "QC Reviewed" by them, and from Smith (1986).
- GLP: Unlikely.
- Year Studies Performed: Unknown. Precedent secondary reference for Smith (1986) is dated 1971. Precedent reference for Hazardous Substances Data Bank (2000) is dated 1995. The precedent reference given in Smith (1986) is the Leo reference that was the source of many of the Log P_{ow} values reported in Verschueren's Handbook (Verschueren, 1996).

RESULTS

Hazardous Substances Data Bank (2000) reported a Log P_{ow} of 1.62. Smith (1986) reported a Log P_{ow} of 2.35 (about a five-fold difference between the two). The precedent references in neither of these two publications appears to be an original publication of the data and the accompanying calculations.

CONCLUSIONS

The numbers from Hazardous Substances Data Bank (2000) and Smith (1986) both indicate that TNG is somewhat more soluble in organic solvents than in water. However, it is not known whether the partition conditions in the two studies cited in these two references, *e.g.*, temperature, organic solvent, organic solvent purity, water hardness, etc. are similar. Nor are the accuracy and precision of the respective analytical methods known at this time. Therefore, a technical comparison of the two numbers is not possible at this time.

DATA QUALITY

Indeterminate at this time.

REFERENCES

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PHYSICAL/CHEMICAL PROPERTIES

5) WATER SOLUBILITY

TEST SUBSTANCE – IDENTITY / PURITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN: 55-63-0)

- Synonyms include almost 100 trade names and other trivial names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is listed as "SNG" (no further identification) in this Handbook section. Number 80066-48-4 is listed in this Handbook section as 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY: Not reported in publication (Ledbury & Frost, 1927).

METHOD

The values reported below from Ledbury & Frost are from the actual reference. All other values reported are from secondary references, as the primary references either were not available or were not given in the secondary references. Ledbury & Frost prepared their solutions by "stirring an excess of filtered TNG with distilled water in a vessel immersed in a thermostat [*sic*], the temperature of which was electrically controlled within 0.25°[C] of the desired point."

"Care was taken to eliminate friction during stirring. After several hours' agitation the undissolved nitroglycerol was allowed to settle out at the temperature of the thermostat and 200 cc of the clear supernatant liquid were withdrawn by means of a calibrated pipette, which previously had been warmed in an incubator to a temperature slightly above that of the experiment. The pipette was finally rinsed out with a little absolute alcohol, the washings being added to the solution under investigation"

"In order to redissolve the nitroglycerol, which separated on cooling, and also to facilitate the subsequent decomposition, absolute alcohol (usually about 20 cc) was added to the solution. About 50 g. of pure stick potash were then introduced and the resulting liquor was refluxed for several hours. [.......]. After cooling, 5 g. of powdered Devarda's alloy were quickly added and the ammonia evolved was determined in the usual manner."

The method was validated by preparing solutions with known weights of TNG in an excessive volume of water. The amounts added were in the range expected from the actual experiments. Recovery was = 96.7%. The experiments were carried out at least twice at each temperature, and the values reported below are the average of the multiple experiments. Variation between the individual determinations never exceeded 2%. In addition, at 40, 50, and 60°C, extra experiments were carried out in which the container with both the TNG and water were heated and stirred in the usual way at temperatures ten degrees higher than the final target temperature. The container and contents were then cooled ten degrees before the aliquot of supernatant solution was withdrawn for hydrolysis and NH_3 analysis. These results never differed by more than 2.9% from the results obtained when the entire experiment was carried out at the target temperature.

GLP**: Given the date of the primary references cited, it is unlikely that the totality of contemp-rary GLPs were followed.**
YEAR STUDIES PERFORMED: See Table below.

RESULTS

TNG SOLUBILITY IN WATER (g/L)

TEMPERATURE (°C)										
15	20	25	30	40	50	60	70	80	INVESTI-GATOR	YEAR PUB'D
1.27	1.38	-	1.5	1.68	1.96	2.36	2.88	3.44	L&F[a]	1927
1.6	-	-	-	-	-	-	-	-	Will	1908
-	1.8	-	-	-	-	-	-	-	Naoum	1924
-	-	-	-	-	2.5	-	-	-	Oehman	1931
-	2.0	-	-	-	-	-	-	-	Lindner	1993
-	-	1.25	-	-	-	-	-	-	Budavari	1996

a. Ledbury & Frost

CONCLUSIONS

The solubilities for TNG in water reported by Ledbury & Frost cover the widest spectrum of temperatures of any such studies found on TNG. The solubility reported for TNG at each temperature is consistent with its immediate neighbors and the entire family of results in that study. When plotted on graph paper with arithmetic coordinates the data points form a smooth continuum. There are no abrupt changes of slope nor any outliers. Higher solubilities have been reported at single temperatures by their contemporaries, as shown in the above table, but the original publications are not available, so reasons for the divergences cannot be established, as mentioned above. Neither Lidner nor Budavari referenced the source of the value each reported, so reasons for the divergences of their numbers also cannot be established. The author of this SIDS document believes the numbers reported by Ledbury & Frost are reliable.

In the opinion of this SIDS document author, Ledbury & Frost allowed adequate time for saturation quantities of TNG to dissolve, had adequate amounts of TNG contacting the water solvent at each temperature to saturate the solvent water, and used conditions that should allow water saturation of TNG to occur at each temperature studied.

DATA QUALITY

The author of this document believes Ledbury & Frost experiments determined the true values of the solubility of TNG in water for the temperature range 15 - 80°C.

REFERENCES

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ENVIRONMENTAL FATE & PATHWAY ELEMENTS
6) PHOTODEGRADATION

TEST SUBSTANCE – IDENTITY / PURITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)
- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for nitroglycerin (TNG) in the National Library of Medicine (NLM) "ChemIDplus" data base. These all are cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

Pure TNG was prepared from a commercial product containing 10 % (w/w) TNG adsorbed on lactose. The TNG was extracted with diethyl ether. The diethyl ether was then removed on a rotary evaporator. Purity was determined by HPLC (See "**ANALYTICAL PROCEDURE**" below).

METHOD

PROCEDURE: Solutions (5.8 ppm; 2.6 x 10^{-5} M) of TNG were prepared in 1) distilled water, 2) filter-sterilized (0.45 micron pore size) water from a eutrophic city pond near the testing laboratory, and 3) river water downstream from a TNG manufacturing plant. The city pond was hundreds of miles from any TNG or explosives manufacturing plant and inside the city limits. The three solutions were placed in 11mm (O.D.) borosilicate test tubes, and then in a rack inclined 60^{o} to the horizon. The rack location was on the laboratory grounds in Menlo Park, CA, in a location that was free of excessive radiation from building walls and windows, and in direct sunlight during daylight hours. The exposure was from Dec. 19, 1979 to Jan. 23, 1980. Dark controls for each water sample were kept at 20^{o}C for the same period.

GLP: Probably. Report does not state, but laboratory carried out toxicology studies for drug companies and the U.S.F.D.A. GLPs were published in the Federal Register in December, 1978.

YEAR: 1979-1980 (Dec. – Jan.).

LIGHT SOURCE: Sun.

RELATIVE INTENSITY *vs*. SUNLIGHT: 1.0

LIGHT ABSORPTION SPECTRUM: Light absorption coefficient (/ Mole / cm) varies from 6 @ 297.5 nm to 0 @ 330.0 nm (3 x 10^{-3} M solution in distilled water and 10 cm cell).

ANALYTICAL PROCEDURE: HPLC using direct aqueous injection. Column: $C_{18\text{-}\mu}$ Bondapak column, 300mm x 4mm; Solvent: acetonitrile / water: 35 / 65; Flow Rate: 2 ml / min.; Detector: U-V @ 254 nm.

REMARKS**: Test Medium, Test Duration, and Dark (Negative) Control are given in the PROCEDURE Section above. There was no positive control.**

RESULTS

The results of the study are shown in the following table:

WATER SOURCE	RATE CONSTANT[1] k_p x 10^7 / sec.	HALF-LIFE[2] (Days)
Distilled	0.69 ± 0.07	116
Downstream from Plant	1.4 ± 0.09	57
Municipal Pond	1.1 ± 0.1	73

1. For sunlight degradation of 5.8 ppm (2.56 x 10^{-5} M) TNG.
2. 24-hr. days with 10 hrs. of sunlight.

QUANTUM YIELD: Not calculated.

INDIRECT PHOTOLYSIS No experiments were carried out.

BREAKDOWN PRODUCTS: Not determined.

CONCLUSIONS

Solar photolysis of TNG is slow in distilled and natural, microorganism-free, surface waters. For unknown reasons, it is almost twice as fast in microorganism-free natural water as it is in distilled water. Further studies were not carried out because of the rapidity of bio-degradation in surface water *vs*. solar photolytic degradation (Comments of Study Director). See Biodegradation Robust Summary.

DATA QUALITY

Very Good. The study was carried out by scientists from a consulting laboratory with an excellent reputation for physical and biological/toxicological studies and should be reliable. **KEY STUDY**.

REFERENCES

CCOHS, 2001. CHEMINDEX CD-ROM. Canadian Center for Occupational; Health and Safety; Issue 2001-4.

Spanggord, R.J., T. Mill, T-W Chou, W.R. Mabey, J.H. Smith, and S. Lee (**1980**). Environmental Fate Studies on Certain Munition Wastewater Constituents. Final Report, Phase II – Laboratory Studies. National Technical Information Service Report ADA 099256.

SIDS_TNG_PhotodegSunFinal

ENVIRONMENTAL FATE & PATHWAYS
7) STABILITY IN WATER

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for nitroglycerin (TNG) in the National Library of Medicine (NLM) "ChemIDplus" data base. These all are cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

The purity of test materials, except that of Capellos, *et al*. (1984) is not known. The material Capellos, *et al*. used was MILSPEC-N246 (1958). Other original references are in Russian or unavailable, and their information has been obtained indirectly through Chemical Abstracts and Spanggord (1980).

METHODS

Capellos, *et al*. (1984) used TNG concentrations of 1.39 – 2.79 x 10^{-3} M in CO_2-free distilled water solutions containing calcium hydroxide at hydroxide ion concentrations of 7.4 x 10^{-3} to 3.02 x 10^{-2} M. Their studies were carried out at "room temperature" (Usually meant to be 25^{o}C). Svetlov, *et al*. (1976) carried out their studies in neutral distilled water at 50-100^{o}C . Rosseel, *et al*. (1974) carried out their studies at 37^{o}C in 1, 2, 3, and 4 N HCl. Spanggord, *et al*. (1980) calculated the following rate constants and half-lives for alkaline, neutral, and acid solutions at the given temperatures based on the information in these three papers.

RESULTS

1. TNG HYDROLYSIS RATE CONSTANTS

Alkaline Conditions: Second order constant = 2.15 X 10^{-2} / mole / sec. at 25^{o}C and pH = 9.

Neutral Conditions: First order constant = 6 x 10^{-8} / sec. at 80^{o}C and pH = 7.

Acid Conditions: First order constant = 1.6×10^{-6} / sec. at 37°C and 1 molar HCl.

2. 1,3-DINITROGLYCERINE & 1-MONONITROGLYCERIN RATE CONSTANTS

Acid Conditions: 3.7×10^{-7} and 2.40×10^{-7} / sec., respectively.

3. CALCULATED HALF-LIVES

Alkaline Conditions (pH 9) @ 25°C: 37 days.

Neutral Conditions (pH 5) @ 80°C: 134 days.

Neutral Conditions (pH 5) @ 25°C: "Years"(Spanggord, 1980)[1].

Acid Conditions (1 molar HCl) @ 37°C**: 5.0 days.**

Acid Conditions (1 molar HCl) @ 25°C: ~ 10 days (Robust Summary author)[1].

Acid Conditions (pH 3) @ 37°C: >100 years.

4. BREAKDOWN PRODUCTS

Alkaline Conditions**. Nitrates, nitrites, oxalates, formates, carbonates, "nitrate esters", "poly-hydroxy polymer" and its nitrate esters.**

Neutral Conditions. Only nitric acid was identified.

Acid Conditions. Products not identified in the abstract.

CONCLUSIONS

At pH values of 3-8 and temperatures around 25°C, TNG would be expected to be quite stable and half-life values probably would be at least one year. At pH values of 9 and above, the half-life would be expected to be a month or longer and the half-life would be expected to vary inversely with the temperature and the pH (Spanggord, 1980).

DATA QUALITY

The quality varies from GOOD to EXCELLENT depending on how heavily you weight the analyses of the hydrolysis products. The experimental work is excellent. This is a **KEY STUDY**.

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1. Assumes that reaction rate will halve for every 10°C decrease in temperature. This is the usual assumption. (Comment of Robust Summary author).

REFERENCES

CCOHS, **2001**. CHEMINDEX CD-ROM. Canadian Center for Occupational Safety & Health; Issue 2001-4.

Capellos, C., W.J. Fisco, C. Ribaudo, V.D. Hogan, J. Campisi, F.X. Murphy, T.C. Castorina, and D.H. Rosenblatt (**1984**). Basic hydrolysis of glyceryl nitrate esters. III. Trinitroglycerin. *Internat. J. Chem. Kinet.*, **16**, 1027 – 1051.

Rosseel, M.T., M.G. Bogaert, D. Keukeleire (**1974**). Quantitative investigation of the acid-catalyzed hydrolysis of glyceryl nitrates. *Bull. Soc. Chim. Belg*., **83**-(5-6) , 211-18; (from *Chem. Abstr*. **81**: 90919f, 1974.

Spanggord, R.J., T. Mill, T-W Chou, W.R. Mabey, J.H. Smith, and S. Lee (**1980**). Environmental Fate Studies on Certain Munition Wastewater Constituents. Final Report, Phase I – Laboratory Studies. National Technical Information Service Report ADA 082372.

Svetlof, B.S., V.P. Shelaputina, and E.P. Malyutina (**1976**). Neutral hydrolysis of poly-nitrates of polyhydric alcohols. *Kinet. Katal*., **17**(2), 508-511; (from *Chem. Abstr*., **85**:62384s, 1976).

ENVIRONMENTAL FATE AND PATHWAYS

8) TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

T SUBSTANCE

- Nitroglycerin (TNG;1,2,3-propanetriol, 1,2,3-trinitrate; CASRN: 55-63-0).

- Synonyms include almost 100 trade names and other trivial names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed in the National Library of Medicine (NLM) "ChemIDplus" data base for TNG. These are all cross-referenced to 55-63-0 in the NLM and Canadian Center for Occupational Health and Safety data bases. The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is listed as "SNG" (no further identification) in this Handbook section. Number 80066-48-4 is listed in this Handbook section as 1,2,3-propanetriol,1,2,3-trinitrate, the systematic name for TNG.

HOD

The EQC software was used to calculate the distribution of TNG in the Air, Water, Soil, and River Sediment Compartments. TNG is a "Type 1" chemical. The TNG water solubility and vapor pressure were obtained from their respective Robust Summaries submitted in this set of Robust Summaries. Half-lives in soil, air, and sediment were obtained from **Howard**, *et al*. (**1991**). Half-lives in surface water were obtained from both **Howard**, *et al*. and from the Water Stability and the Photolysis Robust Summaries that are included in this set of Robust Summaries. Where **Howard**, *et al*. (**1991**) gave a range of half-lives for a compartment, the average value was used for the calculations in the Robust Summary. Distributions (shown below) were calculated using half-lives from the Water Stability Robust Summary at each of two hydrolysis pH values and the half-life from the Photolysis Robust Summary.

ULTS

		TNG DISTRIBUTION (%)			
		AIR	WATER	SOIL	SEDIMENT
LEVEL 1	Howard Values Only	0.261	96.1	3.548	0.079
	Howard + (pH3) H_20 $t_{1/2}$	0.261	96.1	3.548	0.079
	Howard + (pH5) H_20 $t_{1/2}$	0.129	96.2	3.553	0.079
	Howard + photolysis $t_{1/2}$	0.261	96.1	3.548	0.079
LEVEL 2	Howard Values Only	0.261	96.1	3.548	0.079
	Howard + (pH3) H_20 $t_{1/2}$	0.261	96.1	3.548	0.079
	Howard + (pH5) H_20 $t_{1/2}$	0.129	96.2	3.553	0.079
	Howard + photolysis $t_{1/2}$	0.261	96.1	3.548	0.079
LEVEL 3	Howard Values Only	3.992	42.9	53.0	0.027
	Howard + (pH3) H_20 $t_{1/2}$	2.874	58.9	38.2	0.037
	Howard + (pH5) H_20 $t_{1/2}$	0.82	83.8	15.4	0.052
	Howard + photolysis $t_{1/2}$	1.404	79.9	18.6	0.050

CUSSION and CONCLUSIONS

Factoring in the changes due to acid and essentially neutral aqueous hydrolysis and photolysis essentially changed only the water and soil distributions of TNG. And these were changed dramatically only at Level III. This seems to be due principally to the very short aqueous half-life given by Howard, *et al*. (**1991**).

None of the calculations take into account the biodegradation that is known to occur in water, which is presented in the two **BIODEGRADATION** Robust Summaries included in this set of Robust Summaries. Unfortunately, half-lives from those two studies could not be included in this Robust Summary because they disclosed rate constants but no estimated half-lives. (The author of this Robust Summary did not have the physical chemistry background to make that calculation from half-lives. The author also did not have the background to make the compartmental distribution calculation of all these effects combined).

It also is likely that soil and sediment dwelling microorganisms that can metabolize TNG have become established in these compartments containing TNG, just as they have become established in the river water downstream of one of the TNG plants. This would affect the half-lives in soil and sediment. This probably also would affect the compartmentalization of TNG (Opinion of the author of this Robust Summary).

The water stability of TNG at pH 5 in the Water Stability Robust Summary was determined at 80°C. The author of this Fugacity Robust Summary estimated the half-life at 20°C using the rule of thumb that reaction rates are halved for every ten degree Centigrade drop in reaction temperature.

'A QUALITY

As stated in the PHOTODEGRADATION and WATER STABILITY Robust Summaries, the author of this Robust Summary believes the data in those two Summaries varies from Good – Excellent. The author of this Fugacity Robust Summary cannot judge the quality of the half-life data in Howard, *et al*. (1991). However, the aqueous half-life given by Howard, *et al*. seems unreasonably low for a neutral solution of TNG at room temperature based on other data included in this collection of Robust Summaries.

ERENCES

CCOHS, 2001. CHEMINDEX CD-ROM. Canadian Center for Occupational Health and Safety; Issue 2001-4.
Howard, P.H., R.S. Boethling, and W.F. Jarvis. *Handbook of Environmental Degradation Rates* (1991). Lewis Publishers, Chelsea, MI.
TNG Robust Summary on Biodegradation.
TNG Robust Summary on Photodegradation.
TNG Robust Summary on Water Stability.

ENVIRONMENTAL FATE & PATHWAY ELEMENTS
9A) BIODEGRADATION; SELF-INITIATING

TEST SUBSTANCE – IDENTITY / PURITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These all are cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

The test material was isolated from a commercial inerted preparation containing 10% TNG adsorbed on lactose. The source of the test material and its purity are not disclosed in the report. It was obtained either from a military supplier or from a military storage facility. The TNG was isolated by extraction with diethyl ether followed by concentration on a rotary evaporator. Purity was determined by HPLC. Impurities less than 1% (Spanggord, *et al*., (1980). (Source and purity information obtained on 08/19/02 by telecon between the author of this Robust Summary and the primary author of the study report).

METHOD

PROTOCOL. River water was obtained downstream from a TNG process waste discharge from a TNG manufacturing plant. Microorganisms were acclimated, to TNG (10 ppm) over a thirteen-day period. Acclimations were carried out in plain river water, river water with added river sediment, and river water with added yeast extract. They were carried out under aerobic and "microaerophilic" (headspace flushed with nitrogen) conditions.

TNG-acclimated organisms subsequently were inoculated into shaker flasks containing basal-salts medium and 10 – 120 ppm TNG, where they were incubated for 3.6 days. Both the broth and ethyl acetate extracts of it were analyzed for dinitroglycerols (DNGs) and mononitroglycerols MNGs). Biotransformation rate constants also were determined.

TEST TYPE. Aerobic and minimal air conditions.
GLP. Probably. The report does not give GLP status. Study was started in late 1979 or early 1980 at a large, reputable, contract lab. This is one year after U.S. FDA GLP regulations were published in the Federal Register.
YEAR. Report issued Sept., 1980.
CONTACT TIME (TEST). Six hours – five days.
INOCULUM. TNG-acclimated cultures.

REMARKS.

INOCULUM. Organisms aerobically acclimated to TNG.
CONCENTRATIONS of TEST CHEMICAL USED. 2.5 – 120 ppm.
INCUBATION TEMPERATURE. Not reported
DOSING PROCEDURE.
Biotransformation Studies: After acclimation, cells were harvested and transferred to shaker flasks or (at the high concentrations) jar fermenters, containing 10 - 120 ppm TNG in basal salts medium. In the jar fermenters, agitation was provided with a magnetic stir bar instead of shaking.
Rate Constant Studies: For high-population studies, microorganisms were first TNG-acclimated in a jar fermenter for 3 ½ days at 50 ppm TNG. The cells were then harvested and transferred to a small volume of basal salts medium containing 2.5 ppm TNG. The cell concentration was 1.27×10^9 cells / ml.

For studies during the growth phase, after >90% of the 50 ppm of TNG had been metabolized (four days), enough TNG was added to raise the fermenter concentration by five ppm TNG. The cell concentration was 8.1×10^7 cells / ml. In a second growth phase experiment, the cells were allowed to grow for five days before the supplementary 5 ppm TNG was added. The cell concentration in this study was 5.3×10^7 cells / ml.
SAMPLING FREQUENCY. For metabolism studies, analyses were carried out at the end of the incubation period. For rate constant studies, analyses were carried out every two hours for eight hours.
CONTROLS & BLANKS. None reported.
ANALYTICAL METHODS. All analyses were by commercial HPLC. The area under the curve was measured with commercial digital integrator.
Nitrogen Metabolism Studies. **Commercial ion exchange column and 210 nm UV detector.**

Carbon Metabolism Studies. **Commercial C_{18}-Radial Compression column and detection by Thermal Energy Analyzer operated according to the method of Spanggord and Keck (1980).**

RESULTS

During an acclimation period study in jar fermenters, 95% of 120 ppm of TNG in basal salts medium was degraded in 3 ½ days. The cell population increased from 3.7×10^5 to 7.5×10^7.

The pseudo-first-order rate constant (k_{b1}) under high-cell-population conditions was 0.60 / hr. The calculated second-order rate constant was 4.7×10^{-10} ml / cell / hr.

The pseudo-first-order rate constant in the first growth phase study was 8.6×10^{-2} / hr. The calculated second-order rate constant was calculated to be 1.06×10^{-9} ml / cell / hr. The cell count was 8.1×10^7 cells / ml. The pseudo-first-order-rate constant in a second growth phase study was 7.6×10^{-2} / hr. The calculated second order rate constant was 1.43×10^{-9} ml / cell / hr. The cell count was 5.3×10^7 cells / ml.

These investigators were unable to detect any MNG or DNG as metabolites in any of their culture media after the incubation periods with TNG. They used both ultraviolet and Thermal Energy Analytical (TEA) (Spanggord & Keck, 1980) methods. Their analyses indicated that nitrite (inorganic? Comment by author of Robust Summary) was the main metabolite from the nitro groups. These investigators also did not address the question as to whether the microorganisms they used were able to metabolize the glycerol resulting from the denitration of TNG.

CONCLUSIONS

This study and that of Wendt et al. (1978) show that biodegradation of TNG is a widespread capability in the microorganism cosmos.

DATA QUALITY

Good. This is a **KEY** environmental study. However, the report is a little thin on information.

DISCUSSION

It is not surprising to the author of this Robust Summary that:

1. Microorganisms obtained from different sources would metabolize TNG to different degrees and, probably, with different kinetics;
2. That some of these organisms, while they can metabolize it to a degree, cannot use it as a carbon or nitrogen source (See: Robust Summary "Environmental Fate & Pathway Elements / 9) Biodegradation in the Presence of a Carbon Source"); and that
3. Over the years, microbial sports that could metabolize TNG rapidly and completely would predominate in a river below the outfall from a TNG manufacturing plant. Nature works that way.

Nevertheless, it is encouraging to see the probability of microbial degradation of a relatively simple organic chemical validated,

The important conclusion from this study and that of Wendt *et al*. (1978) is that biodegradation, however fast or slow, is a remediation possibility for TNG wastes (Opinion of the author of this Robust Summary).

It is likely that at least one of the microorganisms utilized by Spanggold *et al*. is capable of using glycerol as a carbon source. It is, after all, a relatively simple molecule with no asymmetric atoms (Opinion of the author of this Robust Summary)

REFERENCES

CCOHS, 2001. CHEMINDEX CD-ROM. Canadian Center for Occupational; Health and Safety; Issue 2001-4.

Spanggord, R.J., and R.G. **Keck** (**1980**). Application of high-pressure liquid chromatography and the thermal energy analyzer to analysis of trinitroglycerin and its metabolites in blood. *J. Pharm. Sci*. **69**, 444 - 447. _

Spanggord, R.J., T. Mill, T-W Chou, W.R. Mabey, J.H. Smith, and S. Lee (**1980**). Environmental Fate Studies on Certain Munition Wastewater Constituents. Final Report, Phase II – Laboratory Studies. National Technical Information Service Report ADA 099256.

Wendt, T.M., J.H. Cornell, and A.M. Kaplan (**1978**). Microbial degradation of glycerol nitrates. *Appl. Environ. Microbiol.*, 36 (5), 693-699.

ENVIRONMENTAL FATE & PATHWAY ELEMENTS
9) BIODEGRADATION IN THE PRESENCE OF A CARBON SOURCE

TEST SUBSTANCE – IDENTITY / PURITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for nitroglycerin in the National Library of Medicine (NLM) "ChemIDplus" data base. These all are cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

The TNG solutions for all studies were prepared from a standard aqueous solution containing 980 mg TNG / L (source not given, but probably prepared from a TNG / lactose composite obtained from another U.S. Army site or a U.S. Army contractor. (Comment by author of Robust Summary.) This work was done at the Environmental Protection Lab., U.S. Army, Natick, MA). High Pressure Liquid Chromatography (HPLC) analysis of this solution, under conditions that separated TNG from the two mononitroglycerol, and the two dinitroglycerol possible impurities, showed only one peak and that was identified as TNG by HPLC (Wendt, *et al*. 1978).

METHOD

PROTOCOL. TNG was incubated with either 1) a spectrum of microorganisms obtained from fresh activated sludge from a local domestic sewage treatment plant, or 2) "pure" lines of bacteria obtained by streaking the spectrum from Procedure 1 onto nutrient agar plates and subculturing micro-organisms from the colonies thus obtained. Care was taken to select only colonies for subculturing that each contained organisms with one or more unique morphological characteristics. [This was done in an attempt to get a variety of genetic compositions and thus a variety of enzymatic capabilities (comment of author of Robust Summary)]. All were gram-negative rods, but of varying lengths, lateral thicknesses, and longitudinal wall curvature. Eight "pure" cultures were established in this experiment.

Both aerobic batch shake-flask and continuous-culture conditions were used. Bacterial growth and metabolic denitrofication occurred only in the presence of a carbon source (glucose) for the microorganisms At the end of the culture periods with TNG, the cultures were extracted with organic solvent(s) and the extracts were subjected to TNG and metabolite qualitative analysis by thin-layer chromatography (TLC) and quantitative analysis by HPLC (Wendt, *et al*. 1978).

Reference standards for the two dinitro- and two mononitroglycerol ester metabolites (acronyms = DNG and MNG, respectively) were prepared, purified, and characterized by the methods of Dunstan, *et al*., (1965).

TEST TYPE. Aerobic with and without an extraneous carbon source.
GLP. Unlikely. Paper publication predates publication of even the USFDA GLPs.
YEAR. < Nov. 1978.
CONTACT TIME. Eight – 15 hours for continuous-culture studies in chemostat. Other continuous-culture studies had residence times up to 84 hrs. Residence times not given for batch shake-flask experiments.
INOCULUM. See **PROTOCOL** Section above.

REMARKS.

INOCULA.

Batch Shake-Flask Experiments. Fresh activated sludge from a local domestic sewage treatment plant which, prior to use, was incubated at 30^{o}C for 18 hrs. in nutrient broth, centrifuged and washed three times with sterile buffer solution (0.312 mM KH_2PO_4 solution), and rested for 12 hrs. at 4^{o}C in the sterile buffer.

Continuous-Culture Experiments. Ten ml of aerated activated sludge from the domestic sewage treatment plant were added to both 1) the reaction vessels and 2) the culture vessel of the chemostat. These studies were carried out at room temperature.

Inocula Source. See above.

CONCENTRATIONS of TEST CHEMICAL USED.

Batch Shake-Flask Experiments: 67-68 mg / l.
Continuous-Culture Experiments: 30 – 150 mg / l.

INCUBATION TEMPERATURE.

Batch Shake-Flask Experiments: 30^{o}C.
Continuous-Culture Experiments: "Room Temperature" and 30^{o}C.

DOSING PROCEDURE. Aliquots of sludge organisms culture or "pure" organism cultures were added to nutrient broth. This was followed by measured doses of the aqueous solutions of TNG.

SAMPLING FREQUENCY. At the end of each test period: 8 – 84 hrs, depending on whether the batch shake-flask or continuous-culture procedure was used.

CONTROLS / BLANKS. None.

ANALYTICAL METHODS. After incubation, aliquots of spent growth medium were extracted at Rm. Temp. with either methylene dichloride (CH_2Cl_2) or diethyl ether [$(C_2H_5)_2O$] in a separatory funnel or a liquid / liquid continuous extractor, respectively. The extracts were then concentrated at 40^o C and Rm. Temp., respectively. Aliquots of the condensed extracts were then chromatographed against the known standards previously prepared (See **METHOD** above). .

Qualitative analysis: Ten - 30µ? of the concentrates were spotted on commercial TLC sheets (without fluorescent indicator) and then developed in a commercial chamber using benzene-ethanol (95 / 5, v / v) as the liquid phase. The spots were visualized by a diphenylamine spray (5 %, w / v, in ethanol) followed by U-V exposure (germicidal lamp). Aliquots of controls were run on each chromato-gram.

Quantitative Analysis: HPLC using a commercial 1) liquid chromatograph with a commercial silica column, 2) variable wavelength detector set at 220 nm, and 3) strip chart recorder. The liquid phase was hexane-isopropanol (95 / 5, v / v). Elution peak areas (representing metabolite and reference-standard concen-trations) were determined with a commercial electronic digital integrator.

RESULTS

Continuous-culture multi-stage (chemostat) experiments in the presence of a carbon source (glucose) showed an average 92.2 % reduction in TNG concentration (influent conc. = 150 mg / l) and an overall average reduction of 77.4 % for (TNG + DNG). They did not analyze for MNG in these experiments. Under the same conditions, starting with 30 mg / l of TNG, TLC analysis of the effluent did not reveal any remaining TNG, DNG, or MNG. The authors did not analyze for glycerol.

The investigators also carried out individual shake-flask incubations with the eight "pure" cell lines they had established previously from the waste-treatment sludge (described under **PROTOCOL** above). The test concentration of TNG was 3 mg / l in each flask. Analysis of metabolites was by TLC as above. Two of the lines of microorganisms metabolized all of the TNG to DNGs and MNGs. Two did not metabolize it at all, and four metabolized it partially to DNGs and MNGs. The authors did not say whether a carbon source was added to the media, but since a previous experiment showed that the spectrum of microorganisms in the activated sludge did not metabolize TNG without an added carbon source, glucose probably was added to these eight cultures (Comment by the author of this Robust Summary). Again, the investigators did not analyze for glycerol.

The investigators also concluded that, in addition to TNG not being a carbon source for microorganisms in the domestic sewage treatment sludge, the nitro moiety of TNG was not a nitrogen source for them.

CONCLUSIONS

The investigators clearly have established that TNG is biodegradable to at least glycerol by microorganisms found in sludge from a domestic sewage-treatment plant.

The investigators did not investigate whether glycerol was a suitable carbon source for the microorganisms that biodegraded TNG in the presence of an added carbon source. If it is, the biodegradation should be self-sustaining once it is initiated. However, the question really is moot, since other investigators have found that, as might be expected, microorganisms isolated from river waters downstream from a TNG manufacturing plant can biodegrade TNG without an added carbon source. (See the Robust Summary for Environmental Fate and Pathway Elements / Biodegradation, Self-initiating. This Comment paragraph is by the author of this Robust Summary)

DATA QUALITY

Very Good. This a **KEY** environmental study.

REFERENCES

CCOHS, 2001. CHEMINDEX CD-ROM. Canadian Center for Occupational; Health and Safety; Issue 2001-4.

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ECOTOXICITY ELEMENTS
10) ACUTE TOXICITY TO RAINBOW TROUT

TEST SUBSTANCE – IDENTITY/PURITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases. The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol,1,2,3-trinitrate, the systematic name for TNG.

The Source Stock TNG used in this study was a 10% solution of TNG in absolute ethanol. It was obtained from the U.S. Naval Ordnance Station (NOS) at Indian Head, MD. It was prepared at NOS from a batch of TNG stored in the original, closed, plastic-lined, shipping containers at Rm. Temp. It contained no solvents or stabilizers. The neat TNG had a chemical purity of 99.998% by analysis. It met the NOS's nitration Specification No. N248B (analysis by NOS). Reverse Phase High Pressure Liquid Chromotography (HPLC) analyses at Johns Hopkins University/Applied Physics Laboratory (JHU/APL) detected no impurities. At JHU/APL, the sample was stored in the dark in sealed glass bottles inside "metal flasks" kept at 4°C. The stability of this stock solution was checked periodically during the course of this study by HPLC analysis.

The study reported in this SIDS document was carried out at JHU/APL.

METHOD

Experimental method ASTM Designation E 729-80 (ASTM, 1980) was used to experi-mentally determine the 96 hr. LC_{50} value for the rainbow trout (*Onchorhynchus mykiss*). Flow-through conditions were used for the determination. The rainbow trout were obtained from the Pennsylvania Fish Commission, Reynoldsdale Fish Culture Station, New Paris, PA.

The study was performed as part of an extensive project on environmental toxicity assessments of several explosives components by JHU/APL during the period 1987 – 1991; the report issued in March, 1991. The report does not mention whether GLPs were specifically followed (Burton, *et al*., 1993).

All water quality measurements and TNG concentration measurements were carried out by the High Pressure Liquid Chromatography (HPLC) method of Brüggemann (1983) modified only by the use of 215 nm as the detection wavelength. (See **REMARKS** Section for instrument and operational details and other water characteristics).

All test concentrations and controls were run in duplicate. Concentrations for the determinative evaluations were set with two objectives: 1) a geometric series of five concen-trations with a dilution factor of 0.6 and 2) two of the five concentrations to produce approxi-mately 84% survival and 84% death, respectively, based on results of a preliminary study. At least ten fish were used in each replicate of each test, and each control group.

All concentration data used in the statistical analyses were the arithmetic means of the measured concentrations found in the two replicates for each data point and the controls. The test end point was mortality. The LC_{50} values and their 95% fiducial limits were determined by the probit method, using an EPA statistical program (Stephan, 1978). In all cases, the goodness of fit probability of the data was >0.05.

The 96 hr. LC_{50} for fingerling rainbow trout in this flow-through study was 1.90 mg/L.

REMARKS

The trout were at the fingerling stage (~48 days old) and were acclimated in JHU/APL aerated deep well water at 12(±1)°C for 14-16 days prior to being placed on test. During acclimation the trout were fed ground Salmon Starter Meal (Ziegler Bros., Gardners, PA) 3X daily on weekdays and 2X daily on weekends. Feeding was discontinued 24 hr. prior to test initiation. The photoperiod for all tests was 16 / 8hrs:light / dark by fluorescent lights giving 60-85 foot-candles at the surface of the test chambers.

A secondary stock solution of 1g of TNG / L of absolute ethanol was prepared for making the test solutions. It was prepared by adding the TNG to aerated JHU / APL deep well water, and stirring for 4-8 hrs. It was adjusted to pH 7.0 and contained no stabilizers or other additives. It was then filtered through 0.45µm (pore size) filters and immediately transferred to its

storage container. Its stability was periodically checked by HPLC analysis. It and all subsequent stock / standard solutions were stored in the dark at 4°C in sealed amber glass containers. Standard reference solutions of 10.0, 5.0, 2.5, and 1.0µg / L were prepared for reference from this secondary stock each day analyses were made.

Aqueous samples from the test chambers were immediately filtered to remove particles >0.45µm. If they could not be analyzed immediately, they were stored in amber glass vials fitted with Teflon® (Reg. T.M. E.I. DuPont de Nemours & Co., Inc.) lined caps and analyzed within the 24 hrs. after the sample was taken. Regular Q.C. analyses showed that no to "little" decomposition occur-red during this storage period under these conditions.

Precision and accuracy determinations of the HPLC analytical methods were conducted prior to the start of the bioassays. The analyses were performed with the TNG aliquot dissolved in the neat JHU/APL deep-well water used in the bioassays. (See below for details).

The test chambers were 10L glass aquaria each initially containing 6.4 L of test solution, control solution, or water, and 10 fish. All stock solutions were quantified prior to the start of a test, and each time a new stock solution was prepared during a test. The test solutions were delivered by solenoid-activated proportional diluter systems. They were calibrated 24 hr. prior to the start of a test and were checked and/or recalibrated at least twice daily during each test. The diluters were all-glass, but polyethylene fittings and Tygon® tubing also were used. All diluters were equipped with counters to monitor the cycling rate as well as to ensure proper function of the diluter. Control and test solutions were: 1) held at test temperature, and 2) aerated in their respective headboxes (PE or Fiberglas® tanks) with air from an oil-free compressor. Water samples for HPLC analysis were taken at "Time Zero" and at 24 hr. intervals thereafter. The final sample was taken just before the test was terminated at 96 hrs.

The flow-through volume was 6.0L / 24 hr. day. The maximum wet weight loading of fish was 0.490g / L, determined from the wet weight of the control fish at the end of the experiment.

Test temperature was maintained by placing the test chambers in constant temperature water baths. Temperatures of the baths, test chambers, and one control chamber were monitored continuously and recorded continuously on strip charts. Dissolved oxygen and pH were measured in one replicate of each pair of control and test chambers at Test Times Zero, +24, +48, +72, and +96 hours. Replicate chamber evaluated was alternated for each successive measurement. Conductivity, alkalinity, and total hardness were measured with the same schedule and replicate rotation for one set of control chambers and the high dose test chambers. Routine water quality was measured by procedures from Standard Methods for the Examination of Water & Wastewater (APHA, *et al*., 1985).

In addition, the water as used in all experiments, was analyzed for 43 Priority Pollutants, eight non-priority, but "Hazardous" substances, 26 pesticides, and 13 metals. None of them were detected with detection limits of 2, 2, 0.1µg/L, and <0.2 - <0.0002 mg/L, respectively. The analyses were conducted once at the beginning of the program and again approx. 18 months later.

The test parameters and mortalities are shown in the following table.

Nominal Conc. (mg/L)	0.0	0.0[a]	1.00	1.67	2.78	4.63	7.72
Measured Conc. (mg/L)	0.0	0.0	0.91	1.46	2.47	3.89	6.25
Range (mg/L)	n/a	n/a	0.84-0.97	1.21-1.60	2.10-2.72	3.33-4.44	5.97-6.76
Mortality (24hr.)							
Replicate 1	0	0	0	0	0	5	10
Replicate 2	0	0	0	0	0	6	10
Mortality(48hr.)							
Replicate 1	0	0	0	0	0	7	10
Replicate 2	0	0	0	0	0	9	10
Mortality(72hr.)							
Replicate 1	0	0	0	0	0	10	10
Replicate 2	0	0	0	0	3	10	10
Mortality(96hr.)							

Replicate 1	0	0	0	1	9	10	10
Replicate 2	0	0	0	1	9	10	10

a. Ethanol added at max. conc. used in test chambers

The JHU/APL water quality parameter average values and ranges are shown in the following table.

PARAMETER	AVERAGE VALUE	RANGE
Dissolved Oxygen (mg/L)	9.7	9.0-10.2
PH (Standard Units)	8.3	7.8-8.9
Temperature (°C)	11.5	10.7-11.9
Conductivity (µmhos/cm)	225	210-239
Alkalinity (mg $CaCO_3$/cm)	109	110-135
Hardness (mg/L as $CaCO_3$)	181	176-189

ANALYTICAL INSTRUMENTATION, CONDITIONS & METHODOLOGY

A Waters HPLC system (Waters Associates, Milford, MA) was used for all TNG analyses. Its components were: dual M45 pumps with Model 680 gradient controller; Model 780 data module (integrator); U6K injector; Model 481 variable wavelength UV detector; Z-Module radial compression column system; Model 712 Waters intelligent sample processor (WISP). The column was Waters Bondpak C_{18}-µ.

The operating conditions were:

Mobile Phase: 55% methanol (HPLC grade)**:**45% water (JHU/APL deep well , deionized & glass-distilled.)

Method: Isocratic

Flow Rate: 1.0mL/min.

Detector Setting: 215 nM / 0.02 AUFS

Injection Volume: 2-50µL (analyte conc.-dependent).

Precision of the analytical method was evaluated by injecting a sample three times on each of three separate days. The mean, standard deviation, and relative standard deviation were calculated for each of a low and high concentration. The accuracy of the method was assessed by calculating the percent deviation (percent recovery) of the measurement from the actual quantity injected. The grand arithmetic means of the absolute standard deviations of the two triads of analyses at the two extremes of concentrations evaluated (89.41ng and 4471ng) were ±7.21ng at the low end and ±40ng at the high end. The corresponding grand arithmetic means of the percent relative S.D.s were ±7.71% at the low end and ±0.87% at the high end.

DATA QUALITY

Excellent. This is a KEY STUDY.

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SIDS_TNG_LD50troutAcuteFinal

ECOTOXICITY ELEMENTS
10) ACUTE TOXICITY TO FATHEAD MINNOW

TEST SUBSTANCE – IDENTITY/PURITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occu-pational Health & Safety (CCOHS) toxicological data bases. The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol,1,2,3-trinitrate, the systematic name for TNG.

The Source Stock TNG used in this study was a 10% solution of TNG in absolute ethanol. It was obtained from the U.S. Naval Ordnance Station (NOS) at Indian Head, MD. It was prepared at NOS from a batch of TNG stored in the original, closed, plastic-lined, shipping containers at Rm. Temp. It contained no solvents or stabilizers. The neat TNG had a chemical purity of 99.998% by analysis. It met the NOS's nitration Specification No. N248B (analysis by NOS). Reverse phase High Pressure Liquid Chromotography (HPLC) analyses at Johns Hopkins University/Applied Physics Laboratory (JHU/APL) detected no impurities. At JHU/APL, the sample was stored in the dark in glass bottles inside "metal flasks" kept at 4°C. The stability of this stock solution was periodically checked during the course of this study by HPLC analysis.

The study reported in this SIDS document was carried out at JHU/APL.

METHOD

Experimental method ASTM Designation E 729-80 (ASTM, 1980) was used to experi-mentally determine the 96 hr. LC_{50} value for the fathead minnow (*pimephalus promelas*). Flow-through conditions were used for the determination. The minnows were obtained from the JHU/APL culture maintained at 25(±1)°C in JHU/APL deep well water (see **REMARKS** Section for characterization of the water, fish source and geneology, and JHU/APL husbandry).

All water quality measurements and TNG concentration measurements were carried out by the High Pressure Liquid Chromatography (HPLC) method of Brüggemann (Brüg-gemann,1983) modified only by the use of 215 nm as the detection wavelength. (See **REMARKS** Section for instrument and operational details, and other water characteristics).

All test concentrations and controls were run in duplicate. Concentrations for the determinative evaluations were set with two objectives: 1) a geometric series of five concen-trations with a dilution factor of 0.6 and 2) two of the five concentrations to produce approxi-mately 84% survival and 84% death, respectively, based on results of a preliminary study. A minimum of ten fish were used in each replicate of each test, and each control group.

All concentration data used in the statistical analyses were the arithmetic means of the measured concentrations found in the two replicates for each data point and the controls. The test end point was mortality. The LC_{50} values and their 95% fiducial limits were determined by the probit method, using an EPA statistical program (Stephan, 1978). In all cases the goodness of fit probability of the data was >0.05.

The 96 hr. LC_{50} for fingerling fathead minnows in this flow-through study was 3.58 mg/L.

REMARKS

The fathead minnows were at the juvenile stage when tested. Since they had been bred and raised under the test conditions, no acclimatization was necessary, prior to being placed on test (see below). They were maintained on their regular diet while on test. Feeding was discontinued 24 hr. prior to test initiation. The photoperiod for all tests was 16 / 8hrs:light / dark by fluorescent lights giving 60-85 foot-candles at the surface of the test chambers.

A secondary stock solution of 1g of TNG/L of JHU/APL deep well water was prepared for making the test solutions. It was prepared by adding the TNG to aerated JHU/APL deep well water, stirring for 4-8 hrs., filtering through 0.45µm (pore size) filters and transferring to the storage container. It contained no stabilizers or other additives. Its stability was periodically checked by HPLC analysis. It and all subsequent stock/standard solutions were stored in the dark at 4°C in amber glass containers. Standard reference solutions of 10.0, 5.0, 2.5, and 1.0µg / L were prepared for reference from this secondary stock each day analyses were made.

Aqueous samples from the test chambers were immediately filtered to remove particles >0.45µm. If they could not be analyzed immediately, they were stored in amber glass vials fitted with Teflon® (Reg. T.M. E.I. DuPont de Nemours & Co., Inc.) lined caps and analyzed within the 24 hrs. after the sample was taken. Regular Q.C. analyses showed that no to "little" decomposition occurred during this storage period under these conditions.

Precision and accuracy determinations of the HPLC analytical methods were conducted prior to the start of the bioassays. The analyses were performed with the TNG dissolved in the neat JHU/APL deep-well water used in the bioassays. (See below for details.)

The test chambers were 10L glass aquaria each initially containing 6.4 L of test solution, control solution, or water, and 10 fish. All stock solutions were quantified prior to the start of a test, and each time a new stock solution was prepared during a test. The test solutions were delivered by solenoid-activated proportional diluter systems, calibrated 24 hr. prior to the start of a test. They were checked and/or recalibrated at least twice daily during each test. The diluters were all-glass, but polyethylene fittings and Tygon® tubing also were used. All diluters were equipped with counters to monitor the cycling rate as well as to ensure proper function of the diluter. Control and test solutions were: 1) held at test temperature, and 2) aerated in their respective headboxes (PE or Fiberglas® tanks) with air from an oil-free compressor. Water samples for HPLC analysis were taken at "Time Zero" and at 24 hr. intervals thereafter. The final sample was taken just before the test was terminated at 96 hrs.

The flow-through volume was 4.8L / 24 hr. day. The maximum wet weight loading of fish was 0.030g / L, determined from the wet weight of the control fish at the end of the experi-ment.

Test temperature was maintained by placing the test chambers in constant temperature water baths. Temperatures of the baths, test chambers, and one control chamber were monitored continuously and recorded continuously on strip charts. Dissolved oxygen and pH were measured in one replicate of each pair of control and test chambers at Test Times Zero and +24, +48, +72, and +96 hours. Replicate chamber evaluated was alternated for each successive measurement. Conductivity, alkalinity, and total hardness were measured with the same schedule and replicate rotation for the control chambers and the high dose test chambers. Routine water quality was measured by procedures from Standard Methods for the Examination of Water & Wastewater; APHA, *et al*. (1985).

In addition, the water as used in all experiments was analyzed for 43 Priority Pollutants, eight non-priority, but "Hazardous" substances, 26 pesticides, and 13 metals. None of them were detected with detection limits of 2, 2, 0.1µg/L, and <0.2 - <0.0002 mg/L, respectively. The analyses were conducted once at the beginning of the program and again approximately 18 months later.

The test parameters and mortalities are shown in the following table.

Nominal Conc. (mg / L)	0.0	1.04	1.76	3.68	4.80	8.0
Measured Conc. (mg / L)	0.0	0.94	1.63	3.53	4.51	7.71
Range (mg / L)	n/a	0.90-0.98	1.37-1.82	3.31-3.81	4.27-4.71	7.42-8.13
Mortality (24hr.)						
Replicate 1	0	0	0	0	0	0
Replicate 2	0	0	0	0	0	1
Mortality(48hr.)						
Replicate 1	0	0	0	0	1	1
Replicate 2	0	0	0	0	1	5
Mortality(72hr.)						
Replicate 1	0	0	1	1	2	8
Replicate 2	0	0	0	1	2	7
Mortality(96hr.)						
Replicate 1	0	0	2	3	5	10
Replicate 2	0	1	1	3	7	10

JHU/APL culture procedures for the fathead minnows were similar to those recom-mended by Peltier & Weber (1985). The JHU/APL culture was initiated with fathead minnows obtained from the U.S. EPA Environmental Monitoring and Support Laboratory, Cincinnati, OH. Spawning fish were cultured in Fiberglas® tanks (2.4 x 0.8 x 0.5m; 0.96 m^3 total vol.) containing 0.2 m^3 JHU/APL well water held at 25 (± 1)°C. The spawning adults were fed a diet of frozen brine shrimp (*Artemia* spp.) and TetraMin® staple food (Ramfab Aquarium Products Co., Oak ridge, TN) twice daily. Excess food was removed daily. Five sets of spawning fathead minnows were maintained in the culture tanks at a ratio of 1 M **:** 3 F. Replacement spawners were rotated at *ca*. 3-month intervals. Fry were reared on brine shrimp *nauplii* (<24hr. old) in 19L aquaria at 25 (± 1)°C in JHL/APL deep well water.

No fish were used for testing if they exhibited any signs of disease within the 10 days preceding the start of a test, or if >3% of the hatch died within 48 hrs. preceding the start of the test. The photoperiod was 16 / 8hrs**:**light / dark by fluorescent lights giving 60-85 foot-candles at the surface of the culture vessels.

The JHU/APL water quality parameter average values and ranges are shown in the following table.

PARAMETER	AVERAGE VALUE	RANGE
Dissolved Oxygen (mg/L)	7.4	7.0-7.9
PH (Standard Units)	7.4	7.0-7.9
Temperature (°C)	25.3	24.7-25.7
Conductivity (µmhos/cm)	345	300-390
Alkalinity (mg $CaCO_3$/cm)	100	80-130
Hardness (mg/L as $CaCO_3$)	199	120-240

ANALYTICAL INSTRUMENTATION, CONDITIONS & METHODOLOGY

A Waters HPLC system (Waters Associates, Milford, MA) was used for all NG analyses. Its components were: dual M45 pumps with Model 680 gradient controller; Model 780 data module (integrator); U6K injector; Model 481 variable wavelength UV DETECTOR; Z-Module radial compression column system; Model 712 Waters intelligent sample processor (WISP). The column was Waters Bondpak C_{18}-µ.

The operating conditions were:

Mobile Phase: 55% methanol (HPLC grade)**:**45% water (JHU/APL deep well , deionized & glass-distilled.)

Method: Isocratic

Flow Rate: 1.0mL / min.

Detector Setting: 215 nM / 0.02 AUFS

Injection Volume: 2-50µL (analyte conc.-dependent).

Precision of the analytical method was evaluated by injecting a sample three times on each of three separate days. The mean, standard deviation, and relative standard deviation were calculated for each of a low and high concentration. The accuracy of the method was assessed by calculating the percent deviation (percent recovery) of the measurement from the actual quantity injected. The grand arithmetic means of the absolute standard deviations of the two triads of analyses at the two extremes of concentrations evaluated (89.41ng and 4471ng) were ± 7.21ng at the low end and ± 40ng at the high end. The corresponding grand arithmetic means of the per cent relative S.D.s were ± 7.71% at the low end and ± 0.87% at the high end.

DATA QUALITY

Excellent. This is a KEY STUDY.

GENERAL REMARKS

This study was performed as part of an extensive project on environmental toxicity assessments of several explosives components by JHU/APL during the period 1987 – 1991; the report was issued by JHU/APL in March, 1991 and was issued by the U.S. National Technical Information Service in April, 1993. The report does not mention whether GLPs were specifically followed, but the dates of the work strongly suggest that they were. The complete report for this study is NTIS Document ADA267467 –

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SIDS_TNG_LD50MinnowAcuteFinal

ECOTOXICITY TESTS

REPEATED DOSE TOXICITY

10A) EIGHT DAY DIETARY STUDY IN QUAIL

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These all are cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

Production grade R-TNG dissolved in acetone (50 gm Q.S. 100 ml acetone).

METHOD

METHOD FOLLOWED: Method corresponded to OECD Guideline #205 (Adopted 1984). The OECD Guideline exactly paralleled the protocol for this 1980 study.
TEST TYPE: Dietary administration (continuous dosing).
GLP**: Yes (FDA Guidelines of 1978).**
YEAR: 1980
SPECIES: Bobwhite quail (*Colinus virginianus*).
SEX: Randomized males and females in each pen.
ROUTE of ADMINISTRATION: Oral (daily).
DURATION: Eight days.
DOSE LEVELS:

TREATMENT	PENS	BIRDS / PEN	DIETARY CONCENTRATION (ppm)
Control	5	10	Basal diet only
Lab Std.	5	10	15.9, 25.1, 39.8, 63.1, 100
Test Mtl.	5	10	562, 1000, 1780, 3160, 5620

EXPOSURE PERIOD: First five days.
FREQUENCY OF TREATMENT: Continuous.
CONTROL GROUPS TREATMENT: Same as test groups, except they were fed only basal diet.
POST-EXPOSURE OBSERVATION: Three days
STATISTICAL METHODS: Probit analysis (Finney, 1971).
REMARKS

DEVIATIONS FROM OECD PROTOCOL #452: None.

RESULTS

NOAEL**. 3160 ppm.**
LOAEL. 5620 ppm (decreased food consumption).

There were no deaths attributable to the test material at any of the doses fed. The LD_{50} of the Laboratory Standard (dieldrin), was 32 ppm (28-36), which was within the historical range at the testing laboratory.

CONCLUSIONS

TNG has a very low order of acute toxicity for bobwhite quail. The LD_{50} is greater than 0.5% in their diet when ingested continuously for five days.

QUALITY

Excellent. This is a **KEY** study.

REFERENCES

CCOHS, 2001. CHEMINDEX CD-ROM. Canadian Center for Occupational Health and Safety; Issue 2001-4.
Finney, D.J.(**1971**), *Probit Analysis*, 3rd Edition; Cambridge University Press, Cambridge.

SIDS_TNG_OralQuail5DayFeedFinal

ECOTOXICITY ELEMENTS
11) ACUTE TOXICITY TO THE ALGA *SELENOSTRUM CAPRICORNUTUM*

TEST SUBSTANCE – IDENTITY/PURITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occu-pational Health & Safety (CCOHS) toxicological data bases. The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol,1,2,3-trinitrate, the systematic name for TNG.

The Source Stock TNG used in this study was a 10% solution of NG in absolute ethanol. It was obtained from the U.S. Naval Ordnance Station (NOS) at Indian Head, MD. It was prepared at NOS from a batch of TNG stored in the original, closed, plastic-lined, shipping containers at room temperature. It contained no solvents or stabilizers. The neat TNG had a chemical purity of 99.998% by analysis. It met the NOS's nitration Specification No. N248B (analysis by NOS). Reverse phase High Pressure Liquid Chromotography (HPLC) analyses at Johns Hopkins University / Applied Physics Laboratory (JHU / APL) detected no impurities. At JHU / APL, the sample was stored in the dark in glass bottles inside "metal flasks" kept at 4°C. The stability of this stock solution was checked periodically during the course of this study by HPLC analysis.

A secondary stock solution of 1g of TNG / L of JHU / APL deep well water was prepared for making the test solutions. It was prepared by adding TNG to algal assay medium, stirring for four hours and filter-sterilizing (4.5µm pore size) prior to test initiation. Its stability was periodically checked by HPLC analysis (See **REMARKS** Section for HPLC methodology). It and all subsequent stock/standard solutions were stored in the dark at 4°C in amber glass containers. Standard reference/stock solutions of 10.0, 5.0, 2.5, and 1.0µg/L were prepared from this secondary stock each day analyses were carried out.

METHODS

The EPA TSCA Experimental Method 797.1060 (U.S. EPA, 1985 & 1986) was used to experimentally determine the 96 hr. EC_{50} value for the green alga *S. capricornutum* in the Acute Algal Growth Inhibition Test. The test was carried out under static conditions. The test end point was growth inhibition, as measured by cell "density" determined with a Coulter Counter.

All TNG concentration measurements were carried out by the High Pressure Liquid Chromatography (HPLC) method of Brüggemann (1983) modified only by the use of 215 nm as the detection wavelength. Routine water quality was determined by procedures given in APHA, *et al*. (1985). (See **REMARKS** Section for instrument and operational details and water characteristics).

The starter culture of *S. capricornutum* was obtained from the culture collection at North Texas State Univ., Denton, TX.

Algal test solutions were prepared by dilution of the stock/standard NG solutions with filtered-sterilized (4.5µm pore size) assay media within a sterile transfer room. Test solutions (100ml total vol.) were dispensed into 250ml Delong flasks and inoculated with *S. capricornutum* cells in log growth phase to achieve a density of ˜ 4,000 cells/ml. The flasks were shaken, illuminated, and held at test temperature as described under **REMARKS**.

All test concentrations and controls were run in triplicate. Concentrations for the determinative evaluations were set to give a geometric series of five concentrations with a dilution factor of 0.6.

Growth measurements, the test end-point, (determined by cell density) were made from each replicate at each temperature at 0, 24, 48, 72, and 96 hrs. Algal cell density was determined with one milliliter samples using a Model ZB1 Coulter Counter (Coulter Electronics, Inc., Hialeah, FL). The instrument was calibrated at each use by hemocytometer counts. TNG concentrations were determined at the beginning and end of the test (0 & 96 hrs.)

All concentration data used in the statistical analyses were the arithmetic means of the measured concentrations found in the three replicates, and the controls, for each data point.

The 96 hr. EC_{50} values for growth inhibition were calculated using the "inhibition proportion" technique recommended by Horning & Weber (1985). Because of the very nature of the growth data, the assumptions of the probit analysis were not met in the classical sense. Therefore, the count data were averaged and subsequently converted to "inhibition proportions" using the following formula. $I = C - (T/C) \times 100$, where C = mean growth of controls; and T = mean growth at the given treatment. The probit analysis was then performed on these "inhibition proportions"

REMARKS

The study was performed as part of an extensive project on environmental toxicity assessments of several explosives components by JHU/APL during the period 1987 – 1991; the report issued in March, 1991 (Burton, *et al*., 1991). The report does not mention whether GLPs were specifically followed, but the study dates suggest that they were.

Stock algal cultures were reared in 2.5L Pyrex® culture flasks containing 1L of filter-sterilized double strength "AAP" algal assay medium, with sufficient phosphorus (P) added to achieve a 20:1 Nitrogen(N)**:**P ratio as described in Miller, *et al*. (1978). Cultures were maintained in a constant temperature incubator under constant cool-white fluorescent lights (˜300 foot candles) at a temperature of 20 (± 0.4)°C. on a shaker table oscillating at 100 rpm (± 10). Temperatures of the baths, test chambers, and the control chamber were monitored continuously and recorded continuously on strip charts. Cells in the log growth phase were used to start all tests.

Aqueous samples from the test chambers were immediately filtered to remove particles >0.45µm. If they could not be analyzed immediately, they were stored in amber glass vials fitted with Teflon® (Registered Trade Mark E.I. DuPont de Nemours & CO., Inc.) lined caps and analyzed within the 24 hrs. after the sample was taken. Regular Q.C. analyses showed that no to "little" decomposition occurred during this storage period under these conditions.

Precision and accuracy analyses of the HPLC analytical methods were conducted prior to the start of the bioassays. The analyses were performed with the TNG aliquot dissolved in the neat JHU/APL deep-well water used in the bioassays.

The results of the general water quality analyses of the JHU/APL deep well water as used to make up the test and control solutions are shown in the following table.

PARAMETER	AVERAGE VALUE
PH (Standard Units)	7.8
Alkalinity ($CaCO_3$; mg/L)	156
Hardness (mg/L as $CaCO_3$)	190
Ammonia (as N; mg/L)	0.15
Nitrate (mg/L)	<0.10
Nitrite (mg/L)	<0.10
Total Kjeldahl Nitrogen (mg/L)	0.15
Total Organic Carbon (mg/L)	19

The water, as used in all experiments, also was analyzed for 43 Priority Pollutants, eight non-priority, but "Hazardous" substances, 26 pesticides, and 13 metals. None of them were detected with detection limits of 2, 2, 0.1, and <0.2-<200 µg/L, respectively. The analyses were conducted once at the beginning of the program and again approx. 18 months later.

ANALYTICAL INSTRUMENTATION, CONDITIONS & METHODOLOGY

A Waters HPLC system (Waters Associates, Milford, MA) was used for all NG analyses. Its components were: dual M45 pumps with Model 680 gradient controller; Model 780 data module (integrator); U6K injector; Model 481 variable wavelength UV detector; Z-Module radial compression column system; Model 712 Waters Intelligent Sample Processor (WISP). The column was Waters Bondpak C_{18}-µ.

The operating conditions were:

Mobile Phase: 55% methanol (HPLC grade):45% water (JHU/APL deep well , deionized & glass-distilled.)

Method: Isocratic

Flow Rate: 1.0mL/min.

Detector Setting: 215 nM / 0.02 AUFS

Injection Volume: 2-50µL (analyte conc.-dependent).

Precision of the analytical method was evaluated by injecting a sample three times on three separate days. The mean, standard deviation, and relative standard deviation were calculated for each of a low and high concentration. The accuracy of the method was assessed by calculating the percent deviation (percent recovery) of the measurement from the actual quantity injected. The grand arithmetic means of the absolute standard deviations of the two triads of analyses at the two extremes of concentrations evaluated (89.41ng and 4471ng) were ±7.21ng at the low end and ±40ng at the high end. The corresponding grand arithmetic means of the per cent relative S.D.s were ±7.71% at the low end and ±0.87% at the high end.

RESULTS

The 96 hr EC_{50} for TNG for *S. Capricornutum* (based on cell density) was 1.15 mg/L.

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The test parameters and analytical results are shown in the following table.

Nominal Conc. (mg/L)	0.0	0.22	0.44	0.72	1.20	2.00
Measured Conc. (mg/L)	0.0	0.18	0.37	0.59	1.14	1.89
Range (mg/L)	n/a	0.14-0.21	0.30-0.40	0.51-0.67	1.11-1.20	1.81-2.00
Mean Cell Density (0hr.)						
Replicate 1	4772	4960	4712	4232	5172	4856
Replicate 2	4596	5192	5104	5068	4540	4704
Replicate 3	5200	5016	5032	4784	4960	5076

Mean Cell Density (24hr.)						
Replicate 1	39752	38792	37040	29192	27152	21240
Replicate 2	38440	39160	38032	27240	28192	18664
Replicate 3	40048	39960	35984	29320	26208	20352
Mean Cell Density (48hr.)						
Replicate 1	345080	355620	353400	262740	237380	148140
Replicate 2	364140	359480	355060	256140	227360	145140
Replicate 3	359680	364870	338680	258900	241160	172280
Mean Cell Density (72hr.)						
Replicate 1	796250	770459	742600	611280	498480	295380
Replicate 2	800000	747520	760980	598980	502000	255180
Replicate 3	757280	759080	746700	663100	471500	262080
Mean Cell Density (96hr.)						
Replicate 1	1190000	1167360	1163400	841260	659920	372000
Replicate 2	1180340	1159420	1115160	812400	615620	337840
Replicate 3	1201340	1151420	1146600	802580	576800	343080

DATA QUALITY

Excellent. This is a KEY STUDY.

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SIDS_TNG_EC50AlgaAcuteFinal

ECOTOXICITY ELEMENTS
12) ACUTE TOXICITY TO *CERIODAPHNIA DUBIA*

TEST SUBSTANCE – IDENTITY/PURITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

• Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occu-pational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol,1,2,3-trinitrate, the systematic name for TNG.

PURITY

The Source Stock TNG used in this study was a 10% solution of TNG in absolute ethanol. It was obtained from the U.S. Naval Ordnance Station (NOS) at Indian Head, MD. It was prepared at NOS from a batch of TNG stored in the original, closed, plastic-lined, shipping containers at room temperature. It contained no solvents or stabilizers. The neat TNG had a chemical purity of 99.998% by analysis. It met the NOS's nitration Specification No. N248B (analysis by NOS). Reverse Phase High Pressure Liquid Chromotography (HPLC) analyses at Johns Hopkins University/Applied Physics Laboratory (JHU/APL) detected no impurities. At JHU/APL, the sample was stored in the dark in glass bottles inside "metal flasks" kept at 4°C. The stability of this stock solution was checked periodically during the course of this study by HPLC analysis.

A stock solution of 1g of TNG/l of JHU/APL deep well water was prepared for making the test solutions by adding the appropriate amount of TNG to the water and stirring for 4 – 8 hrs at room temperature. It was then filtered at room temperature through 0.45µm pore size filters and immediately transferred to its storage container. Its stability was periodically checked by HPLC analysis. It and all subsequent stock/standard solutions were stored in the dark at 4°C in amber glass containers. It was then used to make the test concentrations for the *C. dubia* (daphnia) toxicity test. Standard reference solutions also were prepared for reference from this secondary stock each day analyses were made.

METHOD

Experimental method ASTM Designation E 729-80 (ASTM, 1980) was used to experi-mentally determine the 48 hr. LC_{50} value for the daphnia under static conditions. The test solutions were renewed at 24 hrs. The test end point was mortality. The starter culture was obtained from the Center for Lake Superior Environmental Studies, University of Wisconsin – Superior branch.

All water quality measurements and TNG concentration measurements were carried out by the High Pressure Liquid Chromatography (HPLC) method of Brüggemann (Brüggemann, 1983) modified only by the use of 215 nm as the detection wavelength. (See **REMARKS** Section for instrument and operational details and other water characteristics).

All test concentrations and controls were run in duplicate. Concentrations for the determinative evaluations were set with two objectives: 1) a geometric series of five concen-trations with a dilution factor of 0.6 and 2) two of the five concentrations to produce approxi-mately 84% survival and 84% death, respectively, based on results of a preliminary study. At least ten daphnia were used in each replicate of each test, and each control group and were assigned randomly to their test or control group.

All concentration data used in the statistical analyses were the arithmetic means of the measured concentrations found in the two replicates for each data point and the controls. The test end point was mortality. The LC_{50} value was determined by the moving average angle method, using an EPA statistical program (Stephan, 1978).

The study was performed as part of an extensive project on environmental toxicity assessments of several explosives components by JHU/APL during the period 1987 – 1991; the report issued in March, 1991 (Burton, *et al*., 1991). The report does not mention whether GLPs were specifically followed, but the study dates suggest that they were.

REMARKS

The daphnia were neonates (< 6 hrs. old) when placed on test. The stock culture was maintained in 600 m? glass beakers, containing 400 m? of JHU/APL deep well water, enriched with 2µg Se (as Na_2SeO_3)/L as recommended by Winner (personal communication1987; Winner, 1989), and maintained at 25 ± 1°C. The diet consisted of a mixture of Cerophyl® (Cerophyl Laboratories, Inc., Kansas City, MO) and the green alga *Selenastrum capricornutum*, present in the culture at concentrations of 120 µg and 6.7×10^5 cells, per ml, respectively. While on test the daphnia were fed only at test initiation and at the 24 hr. renewal of test solutions. The photoperiod for the stock culture and all tests was 16/8hrs:light/dark. Lighting was by fluorescent lights giving 60-85 foot-candles at the surface of the test chambers.

The test chambers were 50 ml glass beakers, initially containing 30 ml of test solution each. All stock solutions were quantified prior to the start of a test, and each time a new stock solution was prepared during a test. Control and test suspensions were warmed to test temperature before adding the daphnia. While on test, test temperature was maintained by placing the test chambers in an environmental chamber set at 25°C. Temperatures in the environmental chambers were monitored and recorded continuously on strip charts. A temperature probe also was placed in one of the control chambers.

Dissolved oxygen and pH were measured in one replicate of each pair of control and test chambers at Test Times Zero, and +24 hrs for both the original test medium and the 24 hr. replacement medium (Test Times 0, 24, and 48 hrs.). Conductivity, alkalinity, and total hardness were measured on the same schedule for one set of control chambers and the high dose test chambers. Routine water quality was measured by procedures from Standard Methods for the Examination of Water & Wastewater (APHA, *et al*., 1985).

The water used in all exposures was analyzed for 43 Priority Pollutants, eight non-priority, but "Hazardous" substances, 26 pesticides, and 13 metals. None of them were detected with detection limits of 2, 2, 0.1µg/l, and <0.2 - <0.0002 mg/l, respectively. The analyses were conducted once at the beginning of the program and again approx. 18 months later.

Aqueous samples from the test chambers were immediately filtered to remove particles >0.45µm. If they could not be analyzed immediately, they were stored in amber glass vials fitted with Teflon® (Reg. trade mark of E.I. DuPont de Nemours & CO., Inc.) lined caps and analyzed within the 24 hrs. after the sample was taken. Regular Q.C. analyses showed that "no" to "little" decomposition occurred during this storage period under these conditions.

Precision and accuracy analyses of the HPLC analytical methods were conducted prior to the start of the bioassays. The analyses were performed with the TNG aliquot dissolved in the neat JHU/APL deep-well water used in the bioassay. The JHU/APL deep well water was not chlorinated or treated with any other chemicals prior to use. Water quality parameter values and ranges are shown in the following table.

PARAMETER	AVERAGE VALUE	RANGE
Dissolved Oxygen (mg/l)	8.1	7.4-8.5
PH (Standard Units)	8.5	7.9-8.7
Temperature (°C)	24.8	24.6-25.0
Conductivity (µmhos/cm)	409	370-425
Alkalinity (mg $CaCO_3$/l)	159	120-204
Hardness (mg/L as $CaCO_3$)	164	145-186

ANALYTICAL INSTRUMENTATION, CONDITIONS & METHODOLOGY

A Waters HPLC system (Waters Associates, Milford, MA) was used for all TNG analyses. Its components were: dual M45 pumps with Model 680 gradient controller; Model 780 data module (integrator); U6K injector; Model 481 variable wavelength UV DETECTOR; Z-Module radial compression column system; Model 712 Waters intelligent sample processor (WISP). The column was Waters µBondpak C_{18}.

The operating conditions were:

Mobile Phase: 55% methanol (HPLC grade)**:**45% water (JHU/APL deep well , deionized & glass-distilled.)
Method: Isocratic
Flow Rate: 1.0ml/min.
Detector Setting: 215 nM / 0.02 AUFS
Injection Volume: 2-50µl (analyte conc.-dependent).

Precision and accuracy of the analytical method were evaluated by injecting a sample three times on three separate days. The mean, standard deviation, and relative standard deviation were calculated for each of a low and high concentration. The accuracy of the method was assessed by calculating the percent deviation (percent recovery) of the measurement from the actual quantity injected. The grand arithmetic means of the absolute standard deviations of the two triads of analyses at the two extremes of concentrations evaluated (89.41ng and 4471ng) were ±7.21ng at the low end and ±40ng at the high end. The corresponding grand arithmetic means of the per cent relative S.D.s were ±7.71% at the low end and ±0.87% at the high end.

RESULTS

The 48 hr. LC_{50} of TNG for *C. dubia* was 17.83 mg/l; 95% C.L. = 16.48 – 19.51 mg/l.

The test parameters and mortalities are shown in the following table.

Nominal Conc. (mg/l)	0.0	5.80	9.70	16.00	27.00	45.00
Measured Conc. (mg/l)	0.0	5.48	9.45	15.53	26.98	44.80
Range (mg/l)	n/a	5.20-5.70	9.30-9.60	15.20-15.80	26.80-27.10	44.20-45.00
Mortality (24hr.)						
Replicate 1	0	0	0	0	2	10
Replicate 2	0	0	0	0	0	10
Mortality(48hr.)						
Replicate 1	0	0	0	0	3	10
Replicate 2	0	0	0	0	5	10

DATA QUALITY

Excellent. This is a KEY STUDY.

REFERENCES

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Burton, **D.T., S.D. Turley, and G.T. Peters (**1993**). Toxicity of Nitroguanidine, Nitro-glycerin, Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and 2,4,6-trinitrotoluene (TNT) to Selected Freshwater Aquatic Organisms. National Technical Information Service Report ADA267467.**

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SIDS_TNG_LC50DaphniaAcuteFinal

HEALTH ELEMENTS
13)ACUTE TOXICITY
ACUTE ORAL TOXICITY IN MICE

TEST SUBSTANCE – IDENTITY/PURITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)
- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occu-pational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

Production grade TNG adsorbed on lactose to give a product analyzing for 9.72 ± 0.09% (w/w) TNG was used for dosing the mice. It previously had been analyzed by GC (flame ionization detector). No peaks other than those from TNG were seen, with a detection limit (*vs*. TNG = 100%) of 1 % for other components. It also was analyzed for TNG *per se* by the method of Wells (1970). In addition, a sample was extracted with chloroform and an aliquot of the extract was evaporated to yield a residue whose infrared spectrum (between salt plates) was determined. It was identical to that previously reported for TNG (Hayden, *et al*., (1972). For dosing, the lactose mixture was further diluted with peanut oil to give a solution/suspension containing 3.41 % NG (by GC analysis), 31.5% lactose and 65% peanut oil.

METHOD:

METHOD FOLLOWED**: Intragastric intubation.**
TYPE of TEST: Single dose (acute) oral LD_{50}.
GLP: Unlikely. See "YEAR PERFORMED", below.
YEAR PERFORMED: 1975
SPECIES/STRAIN: Mouse (albino, "Swiss") from National Laboratory Co., O'Fallon, MO.
Quarantined and observed for one week prior to test.
SEX: Males and females.
No. per DOSE: Not reported. Probably 10M and 10F / dose

VEHICLE: Peanut oil. See also "PURITY" above.
ROUTE / METHOD of ADMINISTRATION: oral intragastric intubation.

REMARKS

AGE: Not given in report
DOSES: Not given in report
POST-DOSE OBSERVATION: 14 days

RESULTS:

LD_{50} VALUE: The acute oral LD_{50} values and 95% confidence limits in male and female mice were, respectively:
Male: 1,188 mg / kg (1,008-1,352); Female: 1055 mg / kg (895-1178).

The values were calculated by the method of Finney (Finney, 1971).
DEATHS / DOSE: Not reported.
CLINICAL SIGNS: cyanosis, ataxia, depressed respiration, pale eyes/ears/paws/nose/tail. Death usually on Day Zero. Recovery usually complete in 24 hrs.
NECROPSY FINDINGS: No necropsies.
TARGET ORGAN(S): Not identified.

CONCLUSIONS & DATA QUALITY

The report from which the above information was taken was lacking many of the details that were found in the report from which the accompanying robust summary of the rat oral LD_{50} was developed. The group that did the mouse oral LD_{50} study reported in this robust summary also determined the acute oral LD_{50} for rats (See "Acute Oral Toxicity in Rats" Robust Summary).

DATA QUALITY: **Usable.**

REMARKS

The laboratory that carried out this study already had been commissioned to do sub-chronic and chronic ingestion studies with TNG in three species of animals, so they were not asked to do extensive pathology on animals in the acute LD_{50} studies. However, a value for the acute oral LD_{50} in their lab, determined in the mice they would use in the extended studies, was needed in order to set the levels for the next step in the program – subacute studies – so that animals would not be wasted. In view of the subchronic and chronic data that are now available, and for which robust summaries are being submitted, the author of this robust summary does not believe further expenditure of animals in acute oral mouse studies was justifiable. Robust summaries of sub-chronic and chronic studies are being submitted.

This study is reported to illuminate the similar levels of acute oral toxicity observed in two rodent species (rats and mice).

As mentioned above, the laboratory that did the mouse study also determined the oral LD_{50} value of TNG in albino rats. They reported values of: 822 (95% C.L. = 700-953) mg / kg for male rats and 884 (95% C.L. = 763-1055) mg / kg for female rats. This compares with the value for combined males and females of 685 (95% C.L. = 510-940) mg/kg in the rat oral LD_{50} study for which a robust summary also is being submitted in this group of summaries.

Both rat oral LD_{50} values place TNG in the EU CHIP "HARMFUL" category for container labeling (**HSC, 1993**), EPA Category III, and the Hodge & Sterner "Slightly Toxic" category. The mouse oral LD_{50} value also places TNG in the EPA Category III and the Hodge & Sterner "Slightly Toxic" category. If mouse LD_{50} values were permitted under the EU CHIP labeling program, the mouse data also would place TNG in the same toxicity category as the rat data.

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HEALTH ELEMENTS
13) ACUTE TOXICITY
ACUTE ORAL TOXICITY IN RATS

TEST SUBSTANCE – IDENTITY/PURITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)
- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG) in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

The test material was neat TNG, isolated from an ethanolic solution immediately prior to testing. (It is unsafe to transport the undiluted material.) Isolation was by a chemical engineer from sponsor's nearby TNG plant who was familiar with chemistry, physical properties, and handling of TNG. The ethanolic solution was from commercial production (usually 99+ per cent pure). However, sponsor did not supply an analysis.

METHOD

METHOD FOLLOWED**: 16CFR 1500.3(c)(2)(I)[FHSA] (CFR, 2001).**
TYPE of TEST: Single dose (acute) oral LD_{50}.
GLP: Complied with FDA GLP regulations of 6/20/79, proposed TSCA GLP regulations of 5/9/79, and proposed FIFRA GLP regulations of 4/18/80.
YEAR PERFORMED: 1985
SPECIES/STRAIN: Rat (albino); Wistar strain; from Ace Animal Supply.

SEX: Males and females.
No. per DOSE: Five males and 5 females were treated at each dose.
VEHICLE: None. Animals were dosed with undiluted TNG.
ROUTE / METHOD of ADMINISTRATION: oral intragastric intubation.

REMARKS

AGE: The rats were young adults. The male average pre-dose weight was 251.6 ± 14.5 (S.D.)gm. The female average pre-dose weight was 231.2 ± 7.6 (S.D.)gm.

DOSES: Both sexes were dosed on a logarithmic basis; the doses for both sexes were 0.1, 0.3, 1.0, and 3.0 ml/kg; The density of TNG is 1.5931 g/cc @ 20°C (Lide, 1996). Thus, the gravimetric equivalents are: 159, 478, 1593, and 4779mg/kg, respectively. All animals were dosed only once.
POST-DOSE OBSERVATION: Three - 4 hours post-dosing and daily thereafter for an ad-ditional 13 days.
COMMENTS: Pooling the data for males and females is not usual. However, a major differ-ence in oral toxicity between the two sexes would have been observed with this methodology and it serves two purposes in addition to providing an acceptable estimate of the acute oral toxicity: 1) potentially larger group size and 2) conservation of animals. (Comments of author of Robvust Summary). See also final **REMARKS** section in accompanying Mouse Acute Oral Toxicity Robust Summary.

RESULTS:

LD_{50} VALUE: The acute oral LD_{50} value for TNG was calculated for males and females together (Horne, 1956). The value was 0.43 (95% C.L. = 0.32-0.59) ml/kg b.wt.= 685 (510-940) mg/kg.
DEATHS / DOSE: **DAY 0:** 5 males @ each of 4,779mg/kg and 1,593mg/kg; 2 males @ 478 mg / kg
5 females @ 4,779mg / kg and 4 females @ 1593 ml / kg. **DAY 1:** 1 female @ 1,593 mg / kg.
DAYS 2 – 14: (incl.): No further deaths.
CLINICAL SIGNS (See below for codes)**:**

- POST-EXPOSURE DAY 0: (159mg/kg): B (1M), D (1F), E (4M, 4F).
  (478mg/kg): B (1M, 4F), E (4M, 5F), H (2M, 1F), J (4M, 2F), K(1M), L (1M), Z (2M).
  (1,593mg/kg): B (1M, 1F), E (2M, 1F), F (1M), H (1M, 1F), J (1M, 1F), K (1M, 1F), Z (5M, 4F).
  (4,779mg/kg): E (1F), F (1F), Z (5M, 5F).
- POST-EXPOSURE DAY 1: (159 mg/kg): None.
  (478 mg/kg): E (2F).
  (1,593 mg/kg): Z (1F).
- POST-EXPOSURE DAY 2-6, 12, 13: None.
- POST-EXPOSURE DAY 7: (159 mg/kg): C (1M).
- POST-EXPOSURE DAY 8: (159 mg/kg): C (1M), I (1M).
- POST-EXPOSURE DAY 9: (159 mg/kg): C (1M), L (1M).
- POST-EXPOSURE DAY 10: (159 mg/kg): C (1M). L (1M).
- POST-EXPOSURE DAY 11: (159 mg/kg): C (1M).
- POST-EXPOSURE DAY 14: (159mg/kg): A (1F), G (1F).

CLINICAL SIGNS CODES: A = chromodacryorrhea, B = ptosis, C = chromorhinorrhea, D = eyes bluish, E = eyes, ears, and paws bluish, F = coma, G = rales, H = lethargy, I = diarrhea, J = ataxia, K = piloerection, L= anogenital area stained brown.

NECROPSY FINDINGS:

DOSE (mg/kg)	NECROPSY OBSERV'N	MALES					FEMALES				
		1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**

159	Death	S[1]	S	S	S	S	S	S	S	S	S
	Condition		N		N		N	N	N	N	
	EYE: PR[2]										1
	KID: BiHyd			2		2					
	KID: UniHyd	1									
478	Death	D	S	S	D	S	S	S	S	S	S
	Condition			N		N	N	N	N	N	N
	SK: Bl	P			P						
	LU: BiBrown	P			P						
	LI: Dark	3			2						
	KID: UniHyd		1								
	INT: Red	1			1						
1593	Death	D	D	D	D	D	D	D	D	D	D
	AG: Brown				1						
	SK: Bl	P	P	P	P	P	P	P		P	P
	LU: BiCong		1	2		1	2			1	1
	LU: BiBrown	P	P	P	P	P	P	P		P	P
	LI: Dark	3	3	2	3	2	2	2	2	3	2
	KID: UniHyd		2		2						
	AG: Yellow								1		
	ST: Di/F/G								2		
	INT: Red	2	3	3	2	2	3	2	3	2	2
	INT: Yellow	2							2		
	BF: Yellow								2		
4779	Death	D	D	D	D	D	D	D	D	D	D
	NoMo: Red								1	1	
	AG: Brown	1	1					1			
	SK: Bl	P	P	P	P	P	P	P	P	P	P
	LU: BiCong							1	1		
	LU: BiBrown	P	P	P	P	P	P	P	P	P	P
	LI: Dark	3	3	3	3	3	2	3	2	2	2
	KID: BiHyd			2							
	INT: Red	2	3	2	2	2	2	2		1	2
	INT: Yellow	2	2	2	1	2		1	3		

1. **DEATH CODES**: **S** = Sacrificed; **D** = Died.
1. **NECROPSY CODES**: **ORGAN**: **AG** = anogenital area, **BF** = body fat, **INT** = intestines, **KID** = kidneys, **LI** = liver, **LU** = lungs, **NoMo** = nose & mouth, **SK** = skin, **ST** = stomach. **CONDITION**: **Bl** = bluish in bare areas, **Cong =** congested, **Di/F/G** = distended/food/gas, **Hyd** = hydronephrosis, **N** = normal, **P** = present, **PR** = peripheral redness, **OCCURRENCE**: **Bi** = bilateral, **Uni** = unilateral. **SEVERITY**: **1** = slight, **2** = moderate, **3** = pronounced.

TARGET ORGAN(S): Appear to be lungs, liver, and kidneys.

CONCLUSIONS

NG would be considered "Harmful if swallowed" under the EU CHIP Regulations and would be in EPA Category III. The red colored intestines at all but the lowest dose level suggest that it is irritating to the intestinal wall.

DATA QUALITY

VERY GOOD. This is a **KEY STUDY**.

REFERENCES

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GENETIC TOXICITY ELEMENTS

14) GENETIC TOXICITY *IN VIVO*

CHROMOSOME ABERRATIONS
RAT KIDNEY CELLS

(SUBACUTE & SUBCHRONIC ADMINISTRATION)

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)
- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for nitroglycerin (TNG) in the National Library of Medicine (NLM) "ChemIDplus" data base. These all are cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

Production grade TNG adsorbed on lactose to give a product analyzing for 9.72 ± 0.09% (w/w) TNG was used to prepare a stock diet concentrate. The concentrate was analyzed by GC (flame ionization detector). No peaks other than those from TNG were seen, with a detection limit (*vs*. TNG = 100%) of 1 % for other components. It also was analyzed for TNG *per se* by the method of Wells (1970). (This information also was used to guide the weekly preparation of the diets.) In addition, a sample of the diet was extracted with chloroform and an aliquot of the extract was evaporated to yield a residue whose infrared spectrum (between salt plates) was determined. It was identical to that reported for TNG (Hayden, *et al*., (1972).

The diet concentrate was stored in plastic bags which were then stored in five gallon glass jars sealed by wooden plugs covered with aluminum foil. The plugs were held in place by springs. The jars were stored in an underground magazine. Test levels of diet were prepared fresh weekly by dilution of the concentrate with unheated feed and were analyzed after preparation. Control analyses were carried out over eight-day periods to measure evaporation of TNG from the diet under cage conditions during the week. GC also was used for these analyses, but with a ^{63}Ni detector. This information was used to calculate actual dosage received by the rats. Feeders were topped off with fresh diet on the fourth day of each week, and diet in each cage feeder was replaced totally every seven days.

METHOD

METHOD FOLLOWED: Basically OECD Guideline #475, except as noted below. Kidney cells from kidney tissue removed at autopsy were cultured by the trypsinization method of Fernandes (1958). Cultures were maintained in Eagle's medium as modified by Vogt and Dulbecco (1960). Cells were processed for spreading on slides by the method of Moorhead & Newell (1964). Slides were stained with Giemsa stain. Chromosomes were counted and morphological aberrations were examined from photographic negatives of up to 50 metaphase cells.

TEST TYPE: Dietary administration for up to13 weeks. Rats were fed the levels shown below for five weeks. The dietary level of TNG was then raised to give the dietary intakes shown below for the next eight weeks.

GLP: No. Study pre-dated GLPs.

YEAR: 1975 or 1976.

SPECIES: Albino rat.

STRAIN: Charles River CD

SEX: Report does not state if all rat kidneys were from one sex or both sexes.

ROUTE OF ADMINISTRATION: Oral.

DOSE LEVELS: Rats were selected from the highest dose level:

Male average daily intake of NG for first 5 weeks = 59.0 mg/kg/day.
Male average daily intake of NG for weeks 6-13, incl. = 229.5 mg/kg/day.
Female average daily intake of NG for first 5 weeks = 59.3 mg/kg/day.
Female average daily intake of NG for weeks 6-13, incl. = 233.8 mg/kg/day.
Control animals diet was enriched with 25% (w/w) lactose, corresponding to the high dose TNG groups' diets.

EXPOSURE PERIOD: Twenty-four hours/day for up to 13 weeks.

STATISTICAL METHODS: Mean ± standard error.

REMARKS

DEVIATIONS FROM OECD PROTOCOL #475. This study was carried out on rats that had been dosed in their diet for 5 and 13 weeks, respectively. The study also had no positive control. Two hundred cells were evaluated / rat for measuring cell ploidy. Kidney samples from fewer than five rats total were evaluated. The report does not specify whether the kidneys were from males, females, or both. Mitotic indices were not calculated.

RESULTS

GENERAL COMMENTS: This test was part of a suite of tests designed and implemented in the mid-seventies to do a complete toxicological evaluation for nitroglycerin and other munitions chemicals, using Then-contemporary test standards. The results are shown below. Since the rat kidneys were from the highest dose group and the investigators concluded that there appeared to be no effect on the parameters measured, chromosome aberration analyses were not carried out on kidney cells from rats dosed at lower levels. The composite results of the analyses are shown in the tables below.

DAILY DOSE (mg / kg)	No. Of RATS	CHROMOSOME FREQUENCY					TETRAPLOIDS Per 100 CELLS
		=40	41	42	43	= 44	
WKS 1-5							
0	4	4[1]	4	42	1	0	0.59 ± 0.19[2]
Ave. 59.0	3	3	1	44	1	0	0.65 ± 0.33
WKS 6-13							
0	4	4	4	42	1	0	0.59 ± 0.19
Ave. 229.5	4	4	2	43	1	0	0.75 ± 0.25

1. Mean
2. Mean ± standard error.

DOSE (mg / kg)	NUMBER OF RATS	CHROMATID BREAKS & GAPS / 50 CELLS	TRANSLO-CATIONS Per 50 CELLS	TOTAL ABERRATIONS Per 50 CELLS
WKS 1-5				
0	4	1.5 ± 0.3[1]	0.5 ± 0.3	2.0 ± 0.3

Ave. 59.0	3	2.0 ± 0.6	1.0 ± 0.1	2.0 ± 1.3
WKS 6-13				
0	4	1.5 ± 0.3	0.5 ± 0.3	2.0 ± 0.3
Ave. 233.8	4	1.0 ± 0.7	0.5 ± 0.3	1.5 ± 0.9

1. Mean ± standard error.

CONCLUSIONS

The data are indicative, but incomplete by contemporary standards. There is no apparent suggestion of an effect, but the small number of control and test rats evaluated and the lack of information as to the sexes involved make statistical evaluation of the results questionable. The test animal standard errors usually were larger than those for the control animal physical aberration measurements, suggesting more scatter in the test animal measurements, but there does not appear to be any real suggestion of an effect. (Comment of author of this Robust Summary.)

DATA QUALITY

Good (-). (Comment of author of this Robust Summary).

DISCUSSION

Although this study lacked some of the features of the contemporary OECD Guideline, its results are consistent with the other mammalian cell chromosome aberration studies reported in this set of Robust Summaries on TNG. Taken together, 1) the negative results of this study on live intact rats, 2) the negative results from the studies on mammalian lymphocytes, bone marrow cells, and kidney cells (all three types of cells from, variously, rats and dogs), and on ovary cells from hamsters, and 3) the protracted dosing of the animals (except hamsters) from which cells were obtained, make a powerful case for the absence of these types of chromosome aberration activity for TNG. (Conclusion of the author of this Robust Summary.)

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SIDS_TNG_ChromAbsKidRatSubOralFinal

HEALTH ELEMENTS
13) ACUTE TOXICITY

INTRAVENOUS MEDIAN LETHAL DOSE IN RABBITS

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for nitroglycerin (TNG) in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

Purity was not reported (Crandall, 1931).

METHOD

METHOD FOLLOWED: Intravenous.
TYPE of TEST: Single dose (acute).
GLP: No. See date below.
YEAR PERFORMED: 1931
SPECIES/STRAIN: Rabbit; strain unspecified.
SEX & Weight: Sex unspecified; weight "ca. 2 kg".
No. TREATED: Twenty three.
VEHICLE: Aqueous ethanol.
ROUTE / METHOD of ADMINISTRATION: Intravenous; vein not specified. Injections took 5 – 15 seconds.

REMARKS

The dosing emulsion was prepared by diluting an appropriate volume of 10 % ethanolic solution of nitroglycerine with physiological saline to give an emulsion with a final volume of 5 ml. Appropriate volumes of this emulsion were injected. The post-exposure observation period was ten days.

RESULTS

Clinical signs, in order of occurrence, were: respiratory stimulation, reduced heart rate, muscular twitching, tonic convulsions (strychnine type), clonic convulsions, increased heart and respiratory rates, death. Other effects observed (in no regular order) were: constriction of the pupil of the eye followed by dilatation, increased salivation and dacryorrhea, and involuntary urination and defecation. Most deaths occurred within twenty minutes post-dosing, and none occurred after five hours post-dosing. Survivors appeared to recover fully. No animals were necropsied.

CONCLUSIONS

The median lethal intravenous dose of NG in rabbits was 45 mg / kg. By this route, it was rapidly fatal.

DATA QUALITY

The data are "usable". (Comment by author of Robust Summary).

REFERENCES

CCOHS (2001). CHEMINDEX CD-ROM. Canadian Center for Occupational Health and Safety; issue 2001-4.
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HEALTH ELEMENTS
13) ACUTE TOXICITY
DERMAL TOXICITY (LD_{50}) IN RATS

TEST SUBSTANCE – IDENTITY/PURITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)
- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occu-pational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

The test material was neat TNG, isolated from an ethanolic solution immediately prior to testing (It is unsafe to transport the undiluted material). Isolation was by a chemical engineer from sponsor's nearby TNG plant who was familiar with chemistry, physical properties, and handling of TNG. The ethanolic solution was from commercial production (usually 99+ per cent pure). However, sponsor did not supply an analysis.

METHOD

METHOD FOLLOWED**: Laboratory Standard.**
TYPE of TEST: Single dose (acute) dermal LD_{50}.
GLP: Complied with USFDA regulations of 6/20/79 and USEPA regulations of 12/29/83.
YEAR PERFORMED: 1985
SPECIES/STRAIN: Rat (albino); Wistar strain; from Ace Animal Supply.
SEX: Males and females.
NUMBER per DOSE: Ten males and ten females were treated at each dose.
VEHICLE: None. Animals were dosed with undiluted TNG.
ROUTE / METHOD of ADMINISTRATION: Dermal, by repeated application to clipped anterior dorsal area from syringe with ball-tipped stainless steel needle.

REMARKS

AGE: The rats were young adults:

The male average pre-dose weight was 263.6 ± 14.2 (S.D.) gm.

The female average pre-dose weight was 221.5 ± 14.5 (S.D.)gm.

DOSES: Both sexes were dosed at 6.0 ml / kg. The density of TNG is 1.5931 g / cc @ 20°C (Lide,1996). Thus, the gravimetric equivalent is: 9,560mg/kg. All animals were dosed in three equal volume applications separated by a 30 minute absorption period.

POST-DOSE OBSERVATION: Three-4 hours post-dosing and daily thereafter for an additional 14 days.

SURVIVAL: All animals survived.

RESULTS

LD_{50} VALUE: > 6.0 ml / kg (> 9,560 mg / kg).

ADVERSE EFFECTS (Effect codes follow the Table)**:**

DAY	MALES									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
0(pxp)	A,J	A	A	A	A	A,S	A	---	A,S	---
1	B,C,D	A	D	D	D	A,D	A,C	A,C	A,J,Q,C	C
2	B,D	---	D	---	---	---	A,B,D	D	D	D
3	---	---	---	---	---	---	A,B,D	D	---	---
4	G	G	G	G	G	G	G	G	G	G
5	G	G	G	G	G	G	G	G	G	G
6	G	G	G	G	G	G	G	G	G	G
7	G	G	G	G	G	G	G	G	G	G
8	G	---	G	---	---	---	---	G	G	G
9	---	---	G	---	---	---	---	---	---	---
10	NO ADVERSE SIGNS DAYS 10-14, incl. AND DAYS WITH: ---									
DAY	FEMALES									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
0(pxp)	A,J,S	B,S	A,B	A,B,J	B	B	A,B	A,B	A	A,B
1	B,C,D,E	A	A,E	A,E,J	D,E,J	D,J	D,E,F,J	A,E,J	A,E	A,E
2	D	D	---	J,Q	J	D	D,E	D,E	---	---
3	---	D	---	J,Q	J	D	E	D	---	---
4	G	G	G	G,H,J,Q	G,H	G	G,H	G,H	G	G,H
5	G	G	G	G	G,H	G	G,H	G,H	G	G,H
6	G	G	G	G	G,H	G	G,H	G,H	G	G,H
7	G	G	G	G	G,H	G	G,H	G	G	G,H
8	---	---	G	G	G	G	G	G	G	---
9	---	---	G	G	G	G	---	G	G	---
10	---	---	F,G	---	F,G	G	---	---	---	---
11	---	F	F	---	---	G	---	---	---	---
12	---	F	F	---	---	G	---	---	---	---
	NO ADVERSE SIGNS DAYS 13 & 14, incl. AND DAYS WITH: ---									

CLINICAL SIGNS CODES:

A = Mouth wet & brown **E** = Skin bluish **H** = Anogenital area brown

B = Anogenital area wet **F** = Piloerection **J** = Chromodacryorrhea

C = Lacrimation **G** = Body surfaces appear **Q** = Ptosis

D = Body surfaces wet oily & unkempt. **S** = Chromorhinorrhea

CLINICAL SIGNS DISCUSSION: **Clinical signs A, D, and G had essentially equal incidences in males and females. However, signs B, E, H, and J were noted more frequently in females than males. C was reported more frequently in males than females, but the incidences were each so small, that it is doubtful they were significantly different. These sex differences in incidence probably have no significance for humans, medicinally or occupationally.**

NECROPSY RESULTS: **All animals were normal except Males 1 & 10 who had uni-lateral moderate or slight hydronephrosis. Hydronephrosis also was observed in 4 of 5 male rats from the same supplier in an acute oral study (Robust Summary in this set of summaries) in rats from the same supplier in the same year. It also was not seen in females in the latter study.**

TARGET ORGAN(S): None could be identified with the possible exception of the kidney. However, in the Acute Oral study in this strain of rats from the same supplier (see Acute Oral Robust Summary for TNG in Rats), neither the incidence, the severity, nor its unilateral/bilateral occurrence were dose-related, suggesting that it is not test-material related in these rats. Also, in this acute dermal application study, as well as the acute oral study, it occurred only in males. This also suggests it is not test-material related.

CONCLUSIONS

TNG does not appear to be as toxic when absorbed through rat skin as when administered orally. This is evidenced by the lack of dose-related gross effects on internal organs seen following intubation at oral doses that were only 17 and 50% of the dose applied to the rat's skin in this skin absorption study. (See the Acute Oral Rat Study Robust Summary also included in this set of Robust Summaries).

At least two explanations are possible: 1) It is excreted rapidly after penetrating the skin and never reaches an internally toxic level, 2) At the skin diffusion rate, it is metabolized to non-toxic chemicals which are used anabolically or excreted before they reach toxic levels. It has been reported to be absorbed by rat skin from a mixture of 93% TNG / 7% nitrocellulose at the rate of 0.85 mg / sq. cm / hr. (**Gross**, E., *et al*., **1960**).

DATA QUALITY

VERY GOOD. This is a **KEY STUDY**.

REFERENCES

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Gross, E., *et al*. (**1960**). Absorption of Glycerol trinitrate by the skin. *Arch. Toxikol*. 18, 331-334.
Lide, D.R., (Ed.) (**1996**). *CRC Handbook of Chemistry and Physics*. Boca Raton, p. **3**-279, Entry No. 10197.

SIDS_TNG_LD50RatAcuteDermFinal

HEALTH ELEMENTS
13) ACUTE TOXICITY

INHALATION TOXICITY IN ANIMALS

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occu-pational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

RATIONALE FOR NON-PERFORMANCE

Undiluted nitroglycerin is a 'sensitive' high explosive and the danger of a major explosion of TNG vapors or mist in an inhalation study, with resulting major damage to personnel and facility, is too great to consider undertaking such a toxicological study (Comment of author of Robust Summary).

REFERENCES

1. **CCOHS** (**2001**). CHEMINDEX CD-ROM. Canadian Center for Occupational Health and Safety; issue 2001-4

SIDS_TNG_InhalFinal

HEALTH ELEMENTS
13) ACUTE TOXICITY

SKIN IRRITATION IN RABBITS

TEST SUBSTANCE – IDENTITY/PURITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occu-pational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

The test material was neat TNG, isolated from an ethanolic solution immediately prior to testing. (It is unsafe to transport the undiluted material.) Isolation was by a chemical engineer from sponsor's nearby TNG plant who was familiar with chemistry, physical properties, and handling of TNG. The ethanolic solution was prepared from commercial production TNG (usually 99+ per cent pure). However, sponsor did not supply an analysis.

METHOD**: 16CFR 1500.41(FHSA) (CFR, 2001).**

TYPE of TEST: Single dose (acute) Skin Irritation
GLP: Complied with FDA GLP regulations of 6/20/79, proposed GLP TSCA regulations of 5/9/79, and proposed FIFRA GLP regulations of 4/18/80.
YEAR PERFORMED: 1985
SPECIES/STRAIN: Rabbit, New Zealand White from regular supplier.
SEX & Weight: Female; 2.0 – 2.3 kg.
No. TREATED: Six. Animals were observed on-site for seven days before treating.
VEHICLE: None. Animals were dosed with undiluted TNG.
ROUTE / METHOD of ADMINISTRATION: The back & sides of each animal were first clipped free of hair (Sites were reclipped as necessary during the study). The left side of each animal was then abraded with a bent tip needle. Three abrasions, approx. 2-3 cm apart, extending the length of the exposure site were made. The abrasions were deep enough to penetrate the stratum corneum, but not deep enough to produce bleeding. The right side of each animal remained intact. 0.5 ml of undiluted TNG was then applied to each site, for a total of 2.0 ml applied to the skin of each animal. Each application site was then covered with a 2.5 cm square gauze patch which was then secured with adhesive tape. The entire torso was then wrapped with a flexible plastic film (to retard evaporation), which also was secured with adhesive tape. The film and patches were removed after 24 hrs.

Skin reactions were evaluated and recorded at that time, two days later, and six days later. Evaluations (erythema, edema, and eschar formation) were made and scored according to the Draize technique (Draize, 1944). The general health of each also was monitored at each observation.

REMARKS

The protocol differed from 16 CFR 1500.41 in the following ways:

1. Observations of general health also were made at each evaluation time.
2. Animals also were observed & graded on post-exposure day 7.

RESULTS

The Primary Irritation Index (Draize, 1944) was calculated based on the three readings of the two sites on each treated animal. The calculated value was 0.90, indicating mild irritation under the test conditions. Individual component readings were:
Erythema & Eschar (both sites): absent – slight at 24 and 72 hrs.; absent at 7 days. Edema (both sites): absent – slight at 24 hrs.; absent at 72 hrs. & seven days.
Systemic Effects: diarrhea in one animal at 24 hrs.

CONCLUSIONS

Nitroglycerin was a mild, temporary skin irritant to both intact and abraded rabbit skin when evaluated per 1600 CFR 1500.41.

DATA QUALITY

Excellent. In addition, the test method maximized the amount of toxicological infor-mation from a minimal, but adequate, number of test animals. This is a **KEY STUDY**. (Comments by author of Robust Summary).

REFERENCES

1. **CCOHS (2001)**. CHEMINDEX CD-ROM. Canadian Center for Occupational Health and Safety; issue 2001-4.
2. **C**ode of **F**ederal **R**egulations (**2001**), Title 16, Section 1500.42. U.S. Government Printing Office, Washington, DC.
3. **Draize**, J. H., *et al*. (**1944**). Methods for the study of irritation of toxicity of substances applied to the skin and mucous membranes. *J. Pharm. Exp. Ther.*, **82**, 377-390.

HEALTH ELEMENTS
13) ACUTE TOXICITY

EYE IRRITATION IN RABBITS

TEST SUBSTANCE – IDENTITY/PURITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occu-pational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

The test material was neat NG, isolated from an ethanolic solution immediately prior to testing. (It is unsafe to transport the undiluted material.) Isolation was by a chemical engineer from sponsor's nearby NG plant who was familiar with chemistry, physical properties, and handling of NG. The ethanolic solution was from commercial production (usually 99+ per cent pure). However, sponsor did not supply an analysis.

METHOD

METHOD FOLLOWED**: 16CFR 1500.42(FHSA) (CFR, 2001).**
TYPE of TEST: Single dose (acute) Eye Irritation
GLP: Complied with FDA GLP regulations of 6/20/79, proposed TSCA GLP regulations of 5/9/79, and proposed FIFRA GLP regulations of 4/18/80.
YEAR PERFORMED: 1985
SPECIES/STRAIN: Rabbit, New Zealand White from regular supplier.
SEX: Male.
No. per DOSE: Six.
VEHICLE: None. Animals were dosed with undiluted TNG.
ROUTE / METHOD of ADMINISTRATION: Instillation into conjunctival sac of one eye from glass syringe. The eyes (cornea, iris, and conjunctiva) were examined immediately pre-treatment. For treatment, the lower lid was gently pulled away from the eye. 0.1 ml of undiluted TNG was then placed in the sac thus formed. The eyelids were then gently held together briefly ensure adequate distribution. The contralateral eye served as the untreated control. One minute post-treatment, the eyelids of the treated eyes of three rabbits were everted and rinsed with 300 ml of water over a period of three minutes. The treated eye areas of all six rabbits were examined at 1 hr., 24 hrs., 48 hrs., 72 hrs., and 7 days post-treatment.

REMARKS

The protocol differed from 16 CFR 1500.42 in the following ways:

1. The treated eyes of 3/6 rabbits were washed one minute after instillation of the test material.

2. If adverse eye effects, or other adverse effects, were present seven days post treatment, the eyes would be evaluated again at 14 days post-exposure.
3. The general health of the animals also was evaluated each time the eyes were evalu-ated. Any additional clinical signs were recorded and also included in the report of the study. The eyes also were evaluated for any sign of systemic effects, mydriasis, or miosis.
4. Draize (Draize, 1944) numerical scoring was used.

RESULTS

The only effects observed were at one hour post-dosing. They were: Draize rating "1" conjunctival discharge in the treated eyes of 2/3 rabbits whose eyes were not washed and in 3/3 rabbits whose eyes were washed. Rating "1" conjunctival chemosis was seen in 1/3 rabbits whose eyes were washed, and conjunctival redness was seen in another of the rabbits whose eye was washed after treatment. There was no miosis, mydriasis, other ocular effects, or evidence of systemic effects in any of the rabbits treated. All rabbit eyes were normal when examined at all other post-treatment times.

CONCLUSIONS

Undiluted TNG is not an eye irritant as defined by Draize scoring or by 16 CFR1500.42.

DATA QUALITY

Excellent. In addition, the test method maximized the amount of toxicological infor-mation from a minimal, but adequate, number of test animals. This is a **KEY STUDY**. (Comment of author of Robust Summary).

REFERENCES

CCOHS (2001). CHEMINDEX CD-ROM. Canadian Center for Occupational Health and Safety; issue 2001-4.
Code of **F**ederal **R**egulations (**2001**), Title 16, Section 1500.42. U.S. Government Printing Office, Washington, DC.
Draize, J. H., *et al*. (**1944**). Methods for the study of irritation of toxicity of substances applied to the skin and mucous membranes. *J. Pharm. Exp. Ther*., **82**, 377-390.

SIDS_TNG_IrritRabbAcuteEyeFinal

HEALTH ELEMENTS
13) ACUTE TOXICITY

SKIN SENSITIZATION IN GUINEA PIGS

TEST SUBSTANCE – IDENTITY/PURITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occu-pational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

Production grade TNG adsorbed on lactose to give a product analyzing for 9.72 ± 0.09% (w/w) TNG was used in the capsules with which the dogs were dosed once daily. It was analyzed by GC (flame ionization detector). No peaks other than those from NG were seen, with a detection limit (*vs*. TNG = 100%) of 1 % for other components (Lee *et al*., 1975). It also was analyzed for TNG *per se* by the method of Wells (1970). In addition, a sample of the TNG / lactose test material was extracted with chloroform and an aliquot of the extract was evaporated to yield a residue whose infrared spectrum (between salt plates) was determined. It was identical to that reported for TNG (Hayden, *et al*., (1972). For use in this study it was added to peanut oil to give a suspension that was found by G-C analysis to contain 65% peanut oil, 31.5 % lactose and 3.41 % TNG.

METHOD

METHOD FOLLOWED**: Kligman Maximization (Magnusson and Kligman,1969).**
TYPE of TEST: Application to intact skin.
GLP: No. See date below.
YEAR PERFORMED: 1974 - 1975.
SPECIES/STRAIN: Guinea pig (strain not reported), from regular supplier. Observed on-site for one week prior to placing on test.
SEX: Not disclosed in report
No. per TEST: Ten.
VEHICLE: See '**PURITY**' above
ROUTE / METHOD of ADMINISTRATION: Applied to clipped, intact skin, covered with impervious material for duration and number of applications and schedule called for in the Kligman Maximization reference. Challenge and grading per the Kligman Maximization reference. Ten controls received only skin applications of the vehicle: 65% peanut oil and 35 % lactose.

RESULTS

Forty per cent of the guinea pigs were sensitized, as judged by elevated skin irritation response (Lee, et al., 1975).

CONCLUSIONS

NG caused "moderate" skin sensitization in this test and is likely to cause skin sensitization in a small fraction of humans following repeated skin contact.

DATA QUALITY

Excellent. This is a **KEY STUDY**.

REFERENCES

CCOHS (2001). CHEMINDEX CD-ROM. Canadian Center for Occupational Health and Safety; issue 2001-4.
Lee, C-C, J.V. Dilley, J.R. Hodgson, D.O. Helten, W.J. Wiegand, R.N. Roberts, B.S. Anderson, L.M. Halfpep, and L.D. Kurtz (**1975**). Mammalian Toxicity of Munitions Compounds. Report 1. Acute Oral Toxicity, Primary Skin and Eye Irritation, Dermal Sensitization, and Disposition and Metabolism. National technical Information Service Report No. ADB 011150.
Hayden, A. L., *et al*. (**1972**). *Infrared and Ultraviolet Spectra of Some Compounds of Pharmaceutical Interest.* Association of Official Agricultural Chemists, Washington, DC, p. 150.
Magnusson, B. and A.M. **Kligman** (**1969**). The identification of contact allergens by animal assay. The guinea pig maximization test. *J. Invest. Derm*. **52**. 268 – 276.
Wells, C. E., H.M. Miller, and Y.H. Pfabe (**1970**). *J. Assoc. Offic. Analyt. Chem.*, **53**, 579 - 582.

HEALTH ELEMENTS
13A) ACUTE TOXICITY

SKIN SENSITIZATION IN HUMANS

INTRODUCTION

Infrequent reports of human skin sensitization to nitroglycerin have been published. See *e.g*. references Sausker & Frederick (1978), Hardman & Limbird, *et al*. (1996), and Kanerva, *et al*. (1991). In some of these reports, there may have been inadequate appreciation of the possibility that a formulating ingredient may have been the causative agent. However, Sausker & Frederick, and Kanerva, *et al*. appear to have recognized this pitfall and challenged their patients not only with the proper test material, but also with the individual formulating ingredients as well. Although these represent only four patients, TNG *per se* does appear to have the ability to cause skin sensitization in humans, but at a very low incidence; well below that suggested by the guinea pig sensitization test included in this collection of Robust Summaries. This Robust Summary summarizes the Kanerva, *et al*. publication from investigators at the Institute of Dermatology, Institute of Occupational Health, Helsinki, Finland. The incidence of confirmed TNG sensitization seen at the Institute was 4 / 6151 = 0.07 % of the referrals. Three of the sensitizations (2M, 1F) resulted from on-the-job exposure. The fourth (Pt F in **RESULTS** table) was from the use of a transdermal TNG therapeutic product.

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

TNG = 0.01 – 2 % in petrolatum

METHOD:

The skin of the four patients was pricked and the challenge TNG was applied with a Finn Chamber. All applications of the test materials were to the upper back. Challenge materials included the European Standard Series, TNG, ethylene glycol dinitrate, dinitro-toluene, ammonium nitrate, Nitro® ointment and Nitrosid® ointment (both may contain lanolin; Nitrosid® contains isosorbide dinitrate), nitrocellulose, dynamite, and sawdust. All challenges were carried out at the Section of Dermatology, Institute Of Occupational Health, Helsinki, Finland, by section staff. None of the patients had familial atopy, but one was atopic.

TYPE of TEST: Application to pricked skin.
GLP: No. Human clinic setting; No GLPs.
YEARS PERFORMED: 1946 - 1988.
SPECIES/STRAIN: Human
SEX: Two males and two females.
No. per TEST: One.
VEHICLES: Various
ROUTE / METHOD of ADMINISTRATION: See **METHOD** above.

RESULTS

TEST MATERIAL	TNG CONCEN-TRATION (%)	PATIENT 1 RESPONSE (MALE)	PATIENT 2 RESPONSE (MALE)	PATIENT 3 RESPONSE (FEMALE)	PATIENT 4 RESPONSE (FEMALE)
NITRO-GLYCERIN (NG)	2	2+	2+	3+	NT
	0.1	2+	+(?)	3+	NT
	0.05	+	neg	NT	NT
	0.02	2+	NT	NT	1+
	0.01	+	neg	2+	NT
NITROSID OINTMENT	10	NT	NT	3+	NT
NITRO OINTMENT	2	3+	2+	3+	NT
TRANSIDERM NITROPLASTER	?	NT	NT	NT	3+
TRANSIDERM NITROPLASTER PLACEBO	0	NT	NT	NT	neg

NT = Not Tested.
Transderm-Nitro is a registered trademark of CIBA-GEIGY Corp.

CONCLUSIONS

Nitroglycerin appears to be a weaker skin sensitizing chemical in humans than the maximization study in guinea pigs indicates (See Robust Summary for guinea pig skin sensitization studies).

DATA QUALITY

Excellent. This is a **KEY STUDY**.

REFERENCES

CCOHS (2001). CHEMINDEX CD-ROM. Canadian Center for Occupational Health and Safety; issue 2001-4.

Hardman, J.G. and L. L. Limbird (Eds.-in-Chief) (**1996**). Goodman & Gilman's The Phar-macological Basis of Therapeutics (9th Ed.), McGraw-Hill, New York. p.765.

Kanerva, L., R. Laine, R. Jolanki, K. Tarvainen, T. Estlander, and I Helander (**1991**). Occupational allergic contact dermatitis caused by nitroglycerin. *Contact Dermatitis*, **24**, 356-362.

Sausker, W.F. and F.D. **Frederick** (**1978**). Allergic contact dermatitis secondary to topical nitroglycerin. *JAMA*, **239**, 1743-1744.

GENETIC TOXICITY ELEMENTS

14) GENETIC TOXICITY *IN VIVO*
DOMINANT LETHAL ABERRATIONS IN RATS
(SUBCHRONIC DIETARY ADMINISTRATION)

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for nitroglycerin (NG) in the National Library of Medicine (NLM) "ChemIDplus" data base. These all are cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

Pure TNG was used to prepare a stock diet concentrate from feed dried to 0.1 % moisture. The concentrate was analyzed by GC (flame ionization detector). No peaks other than TNG were seen, with a detection limit (*vs*. TNG = 100%) of 1 % for other components. It also was analyzed for TNG *per se* by the method of Wells (1970). (This information was used to guide the weekly preparation of the diets.) In addition, a sample of the diet was extracted with chloroform and an aliquot of the extract was evaporated to yield a residue whose infrared spectrum (between salt plates) was determined. It was identical to that reported for TNG (Hayden, *et al*., (1972).

The diet concentrate was stored in plastic bags which were then stored in five gallon glass jars sealed by wooden plugs covered with aluminum foil. The plugs were held in place by springs. The jars were stored in an underground magazine. Test levels of diet were prepared fresh weekly by dilution of the concentrate with unheated feed and were analyzed after preparation. Control analyses were carried out to measure evaporation of TNG from the diet under cage conditions during the week. GC also was used for these analyses, but with a ^{63}Ni detector. This information was used to calculate actual dosage received by the rats. Feeders were topped off with fresh diet on the fourth day of each week, and diet in each feeder was replaced totally every seven days. The feed for the control rats contained 10 % (w/w) of diet that had been dried to 0.1 % water content.

METHOD

<u>METHOD FOLLOWED</u>: Basically OECD #478. It varied from the OECD protocol in that there was no positive control group, males were dosed for 13 weeks prior to mating, there was no sequential mating, and individual animal data were not reported. Sexually mature virgin females were mated with experienced young adult male rats that had been dosed *via* diet for 13 weeks prior to mating. Dietary levels were selected on the basis of subchronic (13 week) test results.
<u>TEST TYPE</u>: Oral administration in diet.
<u>GLP</u>: Unlikely. Study pre-dated GLP.
<u>YEAR</u>: 1976
<u>SPECIES</u>: Albino rat.
<u>STRAIN</u>: Charles River CD.
<u>SEX</u>: Males & Females
<u>ROUTE OF ADMINISTRATION</u>: Diet
<u>DOSE LEVELS</u>: See Table below for male dose levels Control animals diet was enriched with 25 % (w/w) lactose, corresponding to the lactose in the high dose TNG group's diet.
<u>EXPOSURE PERIOD</u>: Males: Twenty-four hours / day for 13 weeks prior to mating. Females: None.
<u>STATISTICAL METHODS</u>: Mean ± standard error.

RESULTS

REPRODUCTIVE PERFORMANCE OF MALE RATS GIVEN **TNG IN FEED PRIOR TO THE DOMINANT LETHAL MUTATION STUDY**

	PER CENT TNG IN FEED[1]			
	0	**0.01**	**0.1**	**1.0**
MALES Mated[2] Fertile[3]				
	10	10	10	10
	9	8	10	8
FEMALES Receptive[4] Mated[5] Pregnant Complete Resorptions *Corpora Lutea* / Dam Total Implants / Dam Viable Implants / Dam				
	25	25	22	24
	17	15	19	17
	17	14	17	15
	1	0	2	0
	15.0 ± 0.7[6]	13.8 ± 0.8	13.5 ± 0.8	16.1 ± 0.5
	12.4 ± 1.0	12.1 ± 0.8	11.9 ± 1.1	14.1 ± 0.5
	11.4 ± 1.0	11.2 ± 1.0	10.8 ± 1.3	13.0 ± 1.0
INDICES Fertility[7] Gestation[8] Implantation[9] Implant Viability[10]				
	100 (80 – 100)	93 (68 – 100)	89 (67 – 99)	88 (64 – 99)
	94 (71 – 100)	100 (77 – 100)	88 (64 – 99)	100 (78 – 100)
	81 ± 5	89 ± 5	85 ± 6	88 ± 4
	87 ± 6	90 ± 5	81 ± 8	92 ± 5

1. 0.0, 3.04 ± 0.16, 31.5 ± 1.6, and 363 ± 10 mg TNG / kg / day, respectively (mean ± standard error).
2. Exposed to females.
3. Evidence of conception found in at least one female.
4. Proestrous females progressing into estrous overnight.
5. Sperm found in vaginal smear.
1. Mean ± standard error.
2. (Confirmed pregnancies / plug positive females) x 100 (95 % confidence limits).
3. (Pregnancies with viable embryos / confirmed pregnancies) x 100 (95 % confidence limits).
4. (Implants / corporea lutea) x 100 (mean ± standard error).
10. (Viable embryos / implants) x 100 (mean ± standard error).

RESULTS

There was no effect on male fertility. There were no increases in preimplantation or postimplantation losses. There was no effect on the number of impregnations, the number of *corpora lutea*, the total implants / dam, the number of viable implants / dam, or any of the indices that were calculated.

CONCLUSIONS

There was no evidence of a dominant lethal effect. The results also suggest that the reduced litter sizes seen in the high-dose F_1 and F_2 groups in the three-generation reproduction test probably were not due to genetic factors (Opinion of Study Director). The results are consistent with those from all of the other chromosome aberration studies in this submission that were carried out on mammals or mammalian components, including the study on CHO K1 cells which had a positive control. (Conclusions of the author of this SIDS collection of Robust Summaries on NG and / or the Study Director.)

DATA QUALITY

Very Good. (Conclusion of the author of this Robust Summary.)

DISCUSSION

Although this study lacked some of the features of the contemporary OECD Guideline, its results are consistent with the other mammalian cell chromosome aberration studies reported in this set of Robust Summaries on TNG. Taken together, 1) the negative results of this study on live intact rats, 2) the negative results from the studies on mammalian lymphocytes, bone marrow cells, and kidney cells (all three types of cells from, variously, rats and dogs), and on ovary cells from hamsters, and 3) the protracted dosing of the animals (except hamsters) from which cells were obtained, make a powerful case for the absence of these types of chromosome aberration activity for TNG. (Conclusion of the author of this Robust Summary.)

REFERENCES

CCOHS 2001. CHEMINDEX CD-ROM. Canadian Center for Occupational Health and Safety; issue 2001-4.

Ellis, H.V., III, J.H. Hagensen, J.R. Hodgson, J.L. Minor, C-B Hong, E.R. Ellis, J.D. Girvin, D.O. Helton, B.L. Herndon, C-C Lee (**1978**). Mammalian Toxicity of Munition Compounds. Phase III: Effects of Lifetime Exposure. Part II: Trinitroglycerin. Progress Report No. 8. Nat'l. Tech. Info. Serv. Report No. ADA 078746.

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Wells, C. E., H.M. Miller, and Y.H. Pfabe (**1970**). *J. Assoc. Offic. Analyt. Chem.*, **53**, 579 - 582.

SIDS_TNG_DomLethalRatSubDietFinal

GENETIC TOXICITY ELEMENTS

14) GENETIC TOXICITY *IN VIVO*

CHROMOSOME ABERRATIONS
RAT LYMPHOCYTES

(SUBACUTE & SUBCHRONIC DIETARY ADMINISTRATION)

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These all are cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for NG.

PURITY

Production grade TNG adsorbed on lactose to give a product analyzing for 9.72 ± 0.09% (w/w) TNG was used to prepare a stock diet concentrate. The concentrate was ana-lyzed by GC (flame ionization detector). No peaks other than those from TNG were seen, with a detection limit (*vs*. TNG = 100%) of 1 % for other components. It also was analyzed for TNG *per se* by the method of Wells (1970). In addition, a sample and an aliquot of the extract was evaporated to yield a residue whose infrared spectrum (between salt plates) was determined. It was identical to that reported for TNG (Hayden, *et al*., (1972).

The diet concentrate was stored in plastic bags which were then stored in five gallon glass jars sealed by wooden plugs covered with aluminum foil. The plugs were held in place by springs. The jars were stored in an underground magazine. Test levels of diet were prepared fresh weekly by dilution of the concentrate with unheated feed and were analyzed after preparation. Control analyses were carried out to measure evaporation of TNG from the diet under cage conditions during the week. GC also was used for these analyses, but with a ^{63}Ni detector. This information was used to calculate actual dosage received by the rats. Feeders were topped off with fresh diet on the fourth day of each week, and diet in each feeder was replaced totally every seven days. The feed for the control rats contained 10 % (w/w) of diet that had been dried to 0.1 % water content.

METHOD

METHOD FOLLOWED: Basically OECD Guideline #475, except as noted below. Peripheral lymphocytes were obtained immediately prior to necropsy after five and thirteen weeks on test. They were cultured by the method of Moorhead, *et al*. (1960). Cultures were maintained in Eagle's medium as modified by Vogt and Dulbecco (1960). They were stimulated by phytohemagglutin. Cells were processed for spreading on slides by the method of Moorhead & Nowell (1964). Slides were stained with Giemsa stain. Chromosomes were counted and morphological aberrations were examined from photographic negatives of up to 50 metaphase cells.

TEST TYPE: Dietary administration for up to 13 weeks. Rats were fed the levels shown below for the first five weeks. The dietary level of TNG was then raised to give the dietary intakes shown below for the next eight weeks.

GLP: Unlikely. Study pre-dated even USFDA GLPs.

YEAR: 1975– 1976

SPECIES: Albino rat.

STRAIN: Charles River CD.

SEX: Report does not state whether lymphocytes from one sex or both sexes were used. However, since 1) only four males and four females were sacrificed from each group at each of five weeks and thirteen weeks, and 2) four of each sex / group were put on recovery at each of five and thirteen weeks, and 3) each group only started with 16 of each sex, the five rats from each test level that were evaluated for chromosome aberrations at each of five and thirteen weeks had to include rats of both sexes. (Probably three and two). Since only four high dose rats were evaluated at thirteen weeks, it probably was two of each sex. (Speculation of author of this Robust Summary).

ROUTE OF ADMINISTRATION: Diet

DOSE LEVELS: Rats were selected from the highest dose level:

Male average daily intake of TNG for first 5 weeks = 59.0 mg/kg/day.
Male average daily intake of TNG for weeks 6-13, incl. = 229.5 mg/kg/day
Female average daily intake of TNG for first 5 weeks = 59.3 mg/kg/day.
Female average daily intake of TNG for weeks 6-13, incl. = 233.8 mg/kg/day.
Control animals diet was enriched with 25 % (w/w) lactose, corresponding to the lactose in the high dose TNG groups diet.

EXPOSURE PERIOD: Twenty-four hours/day for up to thirteen weeks

STATISTICAL METHODS: Mean ± standard error.

REMARKS

DEVIATIONS FROM OECD PROTOCOL #475. This study was carried out on rats that had been dosed in their diet for 5 and 13 weeks, respectively. The study had no positive control. Two hundred cells were evaluated / rat for measuring cell ploidy. Lymphocytes from only four test rats were evaluated at thirteen weeks. Mitotic indices were not determined.

RESULTS

GENERAL COMMENTS: This test was part of a suite of tests designed and implemented in the mid-seventies to do a complete toxicological evaluation for nitroglycerin and other munitions chemicals, using contemporary test standards. The results are shown below. Since the rat lymphocyte samples were from the highest dose group and the investigators concluded that there appeared to be no effect on the parameters measured, chromosome aberration analyses were not carried out on lymphocytes from rats dosed at the lower levels. The composite results of the analyses are shown in the tables below.

DAILY DOSE (mg / kg)	No. Of RATS	CHROMOSOME FREQUENCY					TETRAPLOIDS Per 100 CELLS
		<76	77	78	79	> 90	
WKS 1-5							
0	5	1[1]	3	44	2	0	0.50 ± 0.16[2]
Ave = 59.0	5	3	3	39	3	1	0.65 ± 0.33
WKS 6-13							
0	5	1	3	44	2	0	0.50 ± 0.16
Ave = 231	4	1	3	44	2	0	0.38 ± 0.23

1. Mean.
2. Mean ± standard error.

DAILY DOSE (mg / kg)	NUMBER OF RATS	CHROMATID BREAKS & GAPS / 50 CELLS	TRANSLO-CATIONS Per 50 CELLS	TOTAL ABERRATIONS Per 50 CELLS
WKS 1- 5				
0	5	1.0 ± 0.3[1]	0.2 ± 0.2	1.2 ± 0.4
1	5	0.2 ± 0.2	0.2 ± 0.2	0.4 ± 0.2
WKS 6-13				
0	5	1.0 ± 0.3	0.2 ± 0.2	1.2 ± 0.4
Ave = 231	4	1.0 ± 0.4	0.3 ± 0.3	1.3 ± 0.6

1. Mean ± standard error

CONCLUSIONS

The data are indicative, but incomplete by contemporary standards. There is no apparent suggestion of an effect. (Comment of author of this Robust Summary and of the investigators.)

DATA QUALITY

Usable. (Comment of author of this Robust Summary.)

Although this study lacked some of the features of the contemporary OECD Guideline, its results are consistent with the other mammalian cell chromosome aberration studies reported in this set of Robust Summaries on TNG. Taken together, 1) the negative results of this study on live intact rats, 2) the negative results from the studies on mammalian lymphocytes, bone marrow cells, and kidney cells (all three types of cells from, variously, rats and dogs), and on ovary cells from hamsters, and 3) the protracted dosing of the animals (except hamsters) from which cells were obtained, make a powerful case for the absence of these types of chromosome aberration activity for TNG. (Conclusion of the author of this Robust Summary.)

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SIDS_TNG_ChromAbsLymphRatSubDietFinal

GENETIC TOXICITY ELEMENTS

14) GENETIC TOXICITY *IN VIVO*

CHROMOSOME ABERRATIONS
RAT BONE MARROW

(CHRONIC DIETARY ADMINISTRATION)

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

Pure TNG was used to prepare a stock diet concentrate from feed dried to 0.1 % moisture. The concentrate was analyzed by GC (flame ionization detector). No peaks other than TNG were seen, with a detection limit (*vs*. TNG = 100%) of 1 % for other components. It also was analyzed for TNG *per se* by the method of Wells (1970). (This information was used to guide the weekly preparation of the diets.) In addition, a sample of the diet was extracted with chloroform and an aliquot of the extract was evaporated to yield a residue whose infrared spectrum (between salt plates) was determined. It was identical to that reported for TNG (Hayden, *et al*., (1972).

The diet concentrate was stored in plastic bags which were then stored in five gallon glass jars sealed by wooden plugs covered with aluminum foil. The plugs were held in place by springs. The jars were stored in an underground magazine. Test levels of diet were prepared fresh weekly by dilution of the concentrate with unheated feed and were analyzed after preparation. Control analyses were carried out to measure evaporation of NG from the diet under cage conditions during the week. GC also was used for these analyses, but with a ^{63}Ni detector. This information was used to calculate actual dosage received by the rats. Feeders were topped off with fresh diet on the fourth day of each week, and diet in each feeder was replaced totally every seven days. The feed for the control rats contained 10 % (w/w) of diet that had been dried to 0.1 % water content.

METHOD

METHOD FOLLOWED: Basically OECD Guideline #475, except as noted below. Femur bone marrow, removed at autopsy, was processed by the method of Eggen (1969). Bone marrow cultures were maintained in nutrient mixture F-12 (HAM). Cells were processed for spreading on slides by the method of Moorhead & Nowell (1964). Slides were stained with Giemsa stain. Chromosomes were counted and morphological aberrations were examined from photographic negatives of up to 50 metaphase cells.

TEST TYPE: Dietary administration for two years.

GLP: Unlikely. Study pre-dated even USFDA GLPs.

YEAR: 1976 – 1978.

SPECIES: Albino rat.

STRAIN: Charles River CD.

SEX: Report does not state if all rat femurs were from one sex or both sexes. However, since an even number each of control and test rats was evaluated, it is probable that half were from each of the two sexes, *i.e.*, three test rats / sex and two control rats per sex. (Comment of author of Robust Summary).

ROUTE OF ADMINISTRATION: Oral.

DOSE LEVELS: Rats were selected from the highest dose levels:

Male average daily intake of TNG for two years = 363 ± 10 mg/kg/day.

Female average daily intake of TNG for two years = 434 ± 11 mg/kg/day.

These were effect levels in rats, causing methemoglobinemia, cholangiofibrosis, hepatocellular carcinomas, testicular interstitial cell tumors, and impaired male fertility.

EXPOSURE PERIOD: Twenty-four hours / day for two years.

STATISTICAL METHODS: Mean ± standard error.

REMARKS

DEVIATIONS FROM OECD PROTOCOL #475. This study was carried out at final necropsy for a two year feeding study and thus the rats were 2+ years old and had been on study for 104 weeks when this study was carried out. The study also had no positive control. Two hundred cells were evaluated / rat for measuring cell ploidy. Chromosome physical evaluations and counts were made from photographic negatives of up to 50 metaphase cells. Mitotic index was not determined.

RESULTS

GENERAL COMMENTS: This test was part of a suite of tests designed and implemented in the mid-seventies to do a complete toxicological evaluation for nitroglycerin and other munitions chemicals, using contemporary test standards. The results are shown below. Since the rat femurs were from the highest dose group, and the investigators concluded that there appeared to be no effect on the marrow parameters measured, chromosome aberration analyses were not carried out on femurs from rats dosed at lower levels. The composite results of the analyses are shown in the tables below.

DAILY DOSE (mg / kg)	NUMBER Of RATS	CHROMOSOME FREQUENCY					TETRAPLOIDS Per 100 CELLS
		=40	41	42	43	= 44	
0	4	1	2	45	1	1	0.62 ± 0.31[1]
= 363 ± 10	6	1	2	44	2	1	0.66 ± 0.17

1. Mean ± standard error.

DAILY DOSE (mg / kg)	NUMBER OF RATS	CHROMATID BREAKS & GAPS / 50 CELLS	TRANSLO-CATIONS Per 50 CELLS	TOTAL ABERRATIONS Per 50 CELLS
0	4	0.6 ± 0.4[1]	0	0.6 ± 0.4
= 363 ± 10	6	1.4 ± 0.3	0	1.4 ± 0.3

1. Mean ± standard error.

CONCLUSIONS

The data are indicative, but incomplete by contemporary standards. There is no strong suggestion of an effect, but the small number of control rats evaluated and the lack of information as to the sexes involved make statistical evaluation of the results questionable. (Comment of author of this Robust Summary.) The apparent increase in chromatid breaks and gaps also was found in the kidney cells (see Chromosome Aberrations, Mouse Kidney Cells Robust Summary). Even if real, the increase may be "of questionable relevance with regard to heritable events of importance to man." (National Research Council, 1975).

DATA QUALITY

Good (-). (Minus due to small number of rats evaluated. Comment of author of this Robust Summary). This is a **KEY STUDY**.

DISCUSSION

Although this study lacked some of the features of the contemporary OECD Guideline, its results are consistent with the other mammalian cell chromosome aberration studies reported in this set of Robust Summaries on NG. Taken together, 1) the negative results of this study on live intact rats, 2) the negative results from the studies on mammalian lymphocytes, bone marrow cells, and kidney cells (all three types of cells from, variously, rats and dogs), and on ovary cells from hamsters, and 3) the protracted dosing of the animals (except hamsters) from which cells were obtained, make a powerful case for the absence of these types of chromosome aberration activity for NG. (Conclusion of the author of this Robust Summary.)

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GENETIC TOXICITY ELEMENTS

14) GENETIC TOXICITY *IN VIVO*

CHROMOSOME ABERRATIONS
RAT KIDNEY CELLS

(CHRONIC DIETARY ADMINISTRATION)

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These all are cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

Pure TNG was used to prepare a stock diet concentrate from feed dried to 0.1 % moisture. The concentrate was analyzed by GC (flame ionization detector). No peaks other than TNG were seen, with a detection limit (*vs*. TNG = 100%) of 1 % for other components. It also was analyzed for TNG *per se* by the method of Wells (1970). (This information was used to guide the weekly preparation of the diets.) In addition, a sample of the diet was extracted with chloroform and an aliquot of the extract was evaporated to yield a residue whose infrared spectrum (between salt plates) was determined. It was identical to that reported for TNG (Hayden, *et al*., (1972).

The diet concentrate was stored in plastic bags which were then stored in five gallon glass jars sealed by wooden plugs covered with aluminum foil. The plugs were held in place by springs. The jars were stored in an underground magazine. Test levels of diet were prepared fresh weekly by dilution of the concentrate with unheated feed and were analyzed after preparation. Control analyses were carried out to measure evaporation of NG from the diet under cage conditions during the week. GC also was used for these analyses, but with a ^{63}Ni detector. This information was used to calculate actual dosage received by the rats. Feeders were topped off with fresh diet on the fourth day of each week, and diet in each feeder was replaced totally every seven days. The feed for the control rats contained 10 % (w/w) of diet that had been dried to 0.1 % water content.

METHOD

METHOD FOLLOWED: Basically OECD Guideline #475, except as noted below. Kidney tissue, removed at autopsy, was processed by Eggen's method (Eggen, 1969). Cells were processed for spreading on slides by the method of Moorhead & Newell (1964). Slides were stained with Giemsa stain. Chromosomes were counted and morphological aberrations were examined from photographic negatives of up to 50 metaphase cells.

TEST TYPE: Dietary administration for two years.

GLP: Unlikely. Study pre-dated even USFDA GLPs.

YEAR: 1976 – 1978.

SPECIES: Albino rat.

STRAIN: Charles River CD

SEX: Report does not state if all rat kidneys were from one sex or both sexes. However, it is likely that there were three control rats of one sex and two of the other. Also, since there were six test rats evaluated, it is likely that there were three test rats from each sex evaluated (Comment of Robust Summary author).

ROUTE OF ADMINISTRATION: Oral

DOSE LEVELS: Rats were selected from the highest dose levels:

Male average daily intake of TNG for two years = 363 ± 10 mg/kg/day.

Female average daily intake of TNG for two years = 434 ± 11 mg/kg/day.

These were effect levels in rats, causing methemoglobinemia, cholangiofibrosis, hepatocellular carcinomas, testicular interstitial cell tumors, and impaired male fertility.

EXPOSURE PERIOD: Twenty-four hours/day for two years.

STATISTICAL METHODS: Mean ± standard error.

REMARKS

DEVIATIONS FROM OECD PROTOCOL #475. This study was carried out at the final necropsy of a two year feeding study and thus the rats were 2+ years old and had been on study for 104 weeks when this study was carried out. The study also had no positive control. Two hundred cells were evaluated / rat for measuring cell ploidy. Chromosome physical evaluations and counts were made from photographic negatives of up to 50 metaphase cells. Mitotic index was not determined.

RESULTS

GENERAL COMMENTS: This test was part of a suite of tests designed and implemented in the mid-seventies to do a complete toxicological evaluation for nitroglycerin and other munitions chemicals, using contemporary test standards. The results are shown below. Since the rat kidneys were from the highest dose group and the investigators concluded that there appeared to be no effect on the parameters measured, chromosome aberration analyses were not carried out on kidney cells from rats dosed at lower levels. The composite results of the analyses are shown in the tables below.

DAILY DOSE (mg / kg)	NUMBER Of RATS	CHROMOSOME FREQUENCY					TETRAPLOIDS Per 100 CELLS
		=40	41	42	43	= 44	
0	5	6	4	38	1	1	0.80 ± 0.30[1]
= 363 ± 10	6	4	4	40	1	1	0.48 ± 0.21

1. Mean ± standard error.

DAILY DOSE (mg / kg)	NUMBER OF RATS	CHROMATID BREAKS & GAPS / 50 CELLS	TRANSLO-CATIONS Per 50 CELLS	TOTAL ABERRATIONS Per 50 CELLS
0	5	1.6 ± 0.5[1]	0	1.6 ± 0.5
= 363 ± 10	6	2.9 ± 0.7	0.1 ± 0.1	3.0 ± 0.7

1. Mean ± standard error.

CONCLUSIONS

The data are indicative, but incomplete by contemporary standards. There is no strong suggestion of an effect, but the small number of control rats evaluated and the lack of information as to the sexes involved make statistical evaluation of the results questionable. (Comment of author of this Robust Summary.) The apparent increase in chromatid breaks and gaps also was found in bone marrow cells (see Chromosome

Aberrations, Rat Bone Marrow Robust Summary). Even if real, the increase may be "of questionable relevance with regard to heritable events of importance to man." (National Research Council, 1975).

DATA QUALITY

Good. (Comment of author of this Robust Summary). This is a **KEY STUDY**.

DISCUSSION

Although this study lacked some of the features of the contemporary OECD Guideline, its results are consistent with the other mammalian cell chromosome aberration studies reported in this set of Robust Summaries on NG. Taken together, 1) the negative results of this study on live intact rats, 2) the negative results from the studies on mammalian lymphocytes, bone marrow cells, and kidney cells (all three types of cells from, variously, rats and dogs), and on ovary cells from hamsters, and 3) the protracted dosing of the animals (except hamsters) from which cells were obtained, make a powerful case for the absence of these types of chromosome aberration activity for NG. (Conclusion of the author of this Robust Summary.)

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Moorhead, P.S. **and** P.C. **Nowell (1964)**. Chromosome Cytology. In *Methods In Medical Research*, H.N. Eisen, Ed. Yearbook Medical Publ., Inc., Chicago, p. 310.

National Research Council, Committee for the Working Conference on Principles of Protocols for Evaluating Chemicals in the Environment, National Academy of Sciences (**1975**). *Principles for Evaluating Chemicals in the Environment*. Washington, DC.

Wells, C.E., C.E., H.M. Miller, and Y.H. Pfabe (**1970**). *J. Assoc. Offic. Analyt. Chem.*, **53**, 579 - 582.

SIDS_TNG_ChromAbsRatKidChronDietFinal

GENETIC TOXICITY ELEMENTS

14) GENETIC TOXICITY *IN VIVO*

CHROMOSOME ABERRATIONS
DOG KIDNEY CELLS

(SUBACUTE & SUBCHRONIC CAPSULE ADMINISTRATION)

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These all are cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

Production grade TNG adsorbed on lactose to give a product analyzing for 9.72 ± 0.09% (w/w) TNG was used in the capsules with which the dogs were dosed. It previously had been analyzed by GC (flame ionization detector). No peaks other than those from TNG were seen, with a detection limit (*vs*. TNG = 100%) of 1 % for other components. It also was analyzed for TNG *per se* by the method of Wells (1970). In addition, a sample was extracted with chloroform and an aliquot of the extract was evaporated to yield a residue whose infrared spectrum (between salt plates) was determined. It was identical to that previously reported for TNG (Hayden, *et al*., (1972).

METHOD

METHOD FOLLOWED: Basically OECD Guideline #475, except as noted below. Kidney cells from kidney tissue removed at necropsy were cultured by the trypsinization method of Fernandes (1958). Cultures were maintained in Eagle's medium as modified by Vogt and Dulbecco (1960). Cells were processed for spreading on slides by the method of Moorhead & Nowell (1964). Slides were stained with Giemsa stain. Chromosomes were counted and morphological aberrations were examined from photographic negatives of up to 50 metaphase cells

TEST TYPE: Once daily oral administration by capsule for up to13 weeks. Dogs initially were dosed at the levels shown below for four weeks. The dosage level of NG was then raised to give the intakes shown below for the next nine weeks.

GLP: Unlikely. Study pre-dated even USFDA GLPs.

YEAR: 1975.

SPECIES: Young beagle dogs from Hazleton Research Animals

SEX: Report does not state if kidneys were from one sex or both sexes See immediate "REMARKS" below.

ROUTE OF ADMINISTRATION: Oral.

DOSE LEVELS: Dogs were selected from the highest dose level:
Male & female daily intake of NG for first 4 weeks = 1 (sic) mg/kg/day.
Male & female daily intake of NG for weeks 5-13, incl. = 5.0 mg/kg/day.
Control animals received empty capsules.

EXPOSURE PERIOD: Twenty-four hours/day for up to thirteen weeks.

STATISTICAL METHODS: Single measurement, or mean of two measurements.

REMARKS

DEVIATIONS FROM OECD PROTOCOL #475. This study was carried out on dogs that had been dosed once daily by capsule for 4 and 13 weeks, respectively. The study had no positive control. One or two dogs were sacrificed at four weeks from each group and two or one at thirteen weeks. The report does not state if kidney samples were from one sex or both sexes. (When two animals were sampled, it would make sense to select one of each sex. When only one was sampled it makes sense to use one sex the first time and the other sex the second time. (Comment of Robust Summary author) Two hundred cells were evaluated / dog for measuring cell ploidy. Mitotic index was not determined.

RESULTS

GENERAL COMMENTS: This test was part of a suite of tests designed and implemented in the mid-seventies to do a complete toxicological evaluation for nitroglycerin and other munitions chemicals, using contemporary test standards. The results are shown below. The dog kidneys evaluated first at thirteen weeks were from the highest dose group. The investigators concluded that there appeared to be no effect on the parameters measured. Therefore, chromosome aberration analyses were not carried out on kidney cells from dogs dosed at lower levels. The composite results of the analyses are shown in the tables below.

DAILY DOSE (mg / kg)	No. Of DOGS	CHROMOSOME FREQUENCY					TETRAPLOIDS Per 100 CELLS
		<76	77	78	79	>90	
WKS 1-4							
0	2	1[1]	1	17	1	0	0.5[1]
1	2	1	2	15	0	0	1.0
WKS 5-13							
0	2	1	1	17	1	0	0.5
5	1	1	3	24	1	0	0.75

1. Single value when only one subject; average when two subjects.

DAILY DOSE (mg / kg)	NUMBER OF DOGS	CHROMATID BREAKS & GAPS / 50 CELLS	TRANSLO-CATIONS Per 50 CELLS	TOTAL ABERRATIONS Per 50 CELLS
WKS 1-4				
0	2	0.0[1]	0.0[1]	0.0[1]
1	2	1.0	1.0	2.0
WKS 5-13				
0	1	0.0	0.0	0.0
5	4	0.0	0.0	0.0

1. Single value when only one subject; average when two or more subjects.

CONCLUSIONS

The data are indicative, but incomplete by contemporary standards. There is no apparent suggestion of an effect, but the small number of (control) dogs evaluated makes statistical evaluation of the results questionable. (Comment of author of this Robust Summary.)

DATA QUALITY

Good (-). (Minus due to small number of control animals). (Comment of author of this Robust Summary.)

DISCUSSION

Although this study lacked some of the features of the contemporary OECD Guideline, its results are consistent with the other mammalian cell chromosome aberration studies reported in this set of Robust Summaries on NG. Taken together, 1) the negative results of this study on live intact dogs, 2) the negative results from the studies on mammalian lymphocytes, bone marrow cells, and kidney cells (all three types of cells from, variously, rats and dogs), and on ovary cells from hamsters, and 3) the protracted dosing of the animals (except hamsters) from which cells were obtained, make a powerful case for the absence of these types of chromosome aberration activity for NG. (Conclusion of the author of this Robust Summary.)

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SIDS_TNG_ChromAbsKidDogSubCapsulFinal

GENETIC TOXICITY ELEMENTS

14) GENETIC TOXICITY *IN VIVO*

CHROMOSOME ABERRATIONS
DOG LYMPHOCYTES

(SUBACUTE & SUBCHRONIC CAPSULE ADMINISTRATION)

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These all are cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

Production grade TNG adsorbed on lactose to give a product analyzing for 9.72 ± 0.09% (w/w) TNG was used in the capsules with which the dogs were dosed once daily. It was analyzed by GC (flame ionization detector). No peaks other than those from TNG were seen, with a detection limit (*vs*. TNG = 100%) of 1 % for other components. It also was analyzed for TNG *per se* by the method of Wells (1970). In addition, a sample of the TNG/lactose test material was extracted with chloroform and an aliquot of the extract was evaporated to yield a residue whose infrared spectrum (between salt plates) was determined. It was identical to that reported for TNG (Hayden, *et al*., (1972).

The diet concentrate was stored in plastic bags which were then stored in five gallon glass jars sealed by wooden plugs covered with aluminum foil. The plugs were held in place by springs. The jars were stored in an underground magazine. Test levels of diet were prepared fresh weekly by dilution of the concentrate with unheated feed and were analyzed after preparation. Control analyses were carried out to measure evaporation of NG from the diet under cage conditions during the week. GC also was used for these analyses, but with a ^{63}Ni detector. This information was used to calculate actual dosage received by the rats. Feeders were topped off with fresh diet on the fourth day of each week, and diet in each feeder was replaced totally every seven days. The feed for the control rats contained 10 % (w/w) of diet that had been dried to 0.1 % water content.

METHOD

METHOD FOLLOWED: Basically OECD Guideline #475, except as noted below. Lymphocytes were obtained from the dog jugular veins immediately prior to necropsy after five and thirteen weeks on test. They were cultured by the method of Moorhead, *et al*. (1960) and stimulated by phytohemagglutin. Cultures were maintained in Eagle's medium as modified by Vogt and Dulbecco (1960). Cells were processed for spreading on slides by the method of Moorhead & Nowell (1964). Slides were stained with Giemsa stain. Chromosomes were counted and morphological aberrations were examined from photographic negatives of up to 50 metaphase cells.

TEST TYPE: Daily capsule administration (one capsule / day) for up to 13 weeks. Dogs initially were dosed the levels shown below for four weeks. The dietary level of NG was then raised to give the dietary intakes shown below for the next 9 weeks.

GLP: Unlikely. Study pre-dated even USFDA GLPs.

YEAR: 1976 – 1978.

SPECIES: Young beagle dogs from Hazleton Research Animals.

SEX: Male and female. Four of each sex were allotted to each of three test groups and one control group.

ROUTE OF ADMINISTRATION: Oral.

DOSE LEVELS: Dogs were selected from the highest dose level:

Male & female daily intake of TNG for first 4 weeks = 1 (sic) mg/kg/day.

Male & female daily intake of TNG for weeks 5-13, incl. = 5.0 mg/kg/day.

Control animals received empty capsules

EXPOSURE PERIOD: Twenty-four hours/day for up to thirteen weeks

STATISTICAL METHODS: Single measurements or mean of two measurements.

REMARKS

DEVIATIONS FROM OECD PROTOCOL #475. This study was carried out on dogs that had been dosed once daily by capsule for 4 and 13 weeks, respectively. The study had no positive control. One female and one male were sacrificed from each group at four weeks and at thirteen weeks. The report does not state if the test dog evaluated at four weeks was male or female. Since only one male and one female were sacrificed after thirteen weeks treatment, the two dogs reported for that time had to be one male and one female. Two hundred cells were evaluated /dog for measuring cell ploidy.

RESULTS

GENERAL COMMENTS: This test was part of a suite of tests designed and implemented in the mid-seventies to do a complete toxicological evaluation for nitroglycerin and other munitions chemicals, using then contemporary test standards. The results are shown below. Since the dog lymphocyte samples were from the highest dose group and the investigators concluded that there appeared to be no effect on the parameters measured, chromosome aberration analyses were not carried out on lymphocytes from dogs dosed at the lower levels. The composite results of the analyses are shown in the tables below.

DAILY DOSE (mg / kg)	No. Of DOGS	CHROMOSOME FREQUENCY					TETRAPLOIDS Per 100 CELLS
		<76	77	78	79	> 90	
WKS 1-4							
0	2	0[1]	2	15	1	0	0.5
1	1	2	4	38	0	0	0.0
WKS 5-13							
0	2	0	2	15	1	0	0.5
5	2	0	3	24	1	0	0.0

1. Single value when only one subject; average when two subjects.

DAILY	NUMBER	CHROMATID	TRANSLO-	TOTAL

DOSE (mg / kg)	OF DOGS	BREAKS & GAPS / 50 CELLS	CATIONS Per 50 CELLS	ABERRATIONS Per 50 CELLS
WKS 1- 4				
0	2	0.5[1]	0.0	0.5
1	1	0.0	1.0	1.0
WKS 5-13				
0	2	0.5	0.0	0.5
5	2	0.5	0.0	0.5

1. Single value when only one subject; average when two subjects.

CONCLUSIONS

The data are indicative, but incomplete by current contemporary standards. There is no apparent suggestion of an effect, but the small number of dogs evaluated and the lack of information as to the sexes involved make statistical evaluation of the results questionable. (Comment of author of this Robust Summary.)

DATA QUALITY

Usable, but inadequate number of subjects. (Comment of author of this Robust Summary.)

DISCUSSION

Although this study lacked some of the features of the contemporary OECD Guideline, its results are consistent with the other mammalian cell chromosome aberration studies reported in this set of Robust Summaries on TNG. Taken together, 1) the negative results of this study on live intact dogs, 2) the negative results from the studies on mammalian lymphocytes, bone marrow cells, and kidney cells (all three types of cells from, variously, rats and dogs), and on ovary cells from hamsters, and 3) the protracted dosing of the animals (except hamsters) from which cells were obtained, make a powerful case for the absence of these types of chromosome aberration activity for TNG. (Conclusion of the author of this Robust Summary.)

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GENETIC TOXICITY ELEMENTS

15) GENETIC TOXICITY *IN VITRO*

MUTAGENICITY IN CHINESE HAMSTER OVARY (CHO-K1) CELLS

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These all are cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

Pure TNG adsorbed on lactose to give a product analyzing for 9.72 ± 0.09% (w/w) TNG by the method of Wells (1970) was used in this study. In addition, 1) a chloroform extract of the concentrate was analyzed by GC (flame ionization detector). No peaks other than those from TNG were seen, with a detection limit (*vs*. TNG = 100%) of 1 % for other components; and 2) an aliquot of the extract was evaporated to yield a residue whose infrared spectrum (between salt plates) was identical to that reported for TNG (Hayden, *et al*., 1972).

METHOD

METHOD FOLLOWED: Basically OECD # 476, with the following deviations – No metabolic activation, two dose levels, no negative control. Wild type cells (Kao and Puck, 1968) capable of growth in both a minimal and enriched medium were exposed. Concentrations were selected from a single cell survival curve obtained according to the method of Puck and Kao (1967). Potential mutants were isolated by the BudR-visible light technique and confirmed by plating the cells in both aforesaid media. A mutant was defined as having growth capability in only the enriched medium. The positive control was ethylmethanesulfonate (Kao and Puck, 1969).

TEST TYPE Mutagenicity.
GLP No. See date below.
YEAR 1975.
SPECIES / STRAIN Wild type Chinese hamster ovary cells.
ROUTE of ADMINISTRATION Whole cell incubation.
DURATION / EXPOSURE PERIOD (not reported).

RESULTS

MUTATION FREQUENCY OF CHO-K1 CELLS TREATED WITH TNG				
TREATMENT	MEAN LETHAL CONCENTRATION (D_0) (µg / ml)	CONCENTRATION TESTED (µg / ml)	SURVIVAL (%)	SEE FOOTNOTE 1
Nitroglycerin	47.0	50.0	35	0

		144.8	1	0
Ethyl methanesulfonate	67.5	124.0	15	2870[2]

1. Summed mutation frequency, per D_0 per cell, for all loci tested (x 10^{-6}).
2. Corrected for loss during mutant isolation.

CONCLUSIONS

This test was part of a suite of tests designed and implemented in the mid-seventies to carry out a complete toxicological evaluation for nitroglycerin and other munitions chemicals, using then-contemporary standards. No mutagenic effects were evident from TNG under the conditions of this test.

DATA QUALITY

Good. (Comment of the author of this Robust Summary). This is a **KEY STUDY**.

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SIDS_TNG_CHO_MutFinal

GENOTOXICITY ELEMENTS

15) *IN VITRO* MUTAGENICITY TO *SALMONELLA TYPHIMURIUM* MUTANTS

TEST SUBSTANCE – IDENTITY / PURITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for nitroglycerin (TNG) in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occu-pational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

REMARKS

The material as received by the testing laboratory was a 5% (w/w) solution of TNG in dimethylsulfoxide (DMSO). The test solutions were prepared by diluting this stock solution with DMSO.

METHOD

-

Although this test was carried out in 1980, the protocol tracked the bacterial reverse mutation assay as described in OECD Guideline No. 471 as issued on 21 July, 1997, with one exception. The exception is described in the following **REMARKS** section. The plate incorporation method was followed. The starter cultures of the five auxotrophs used were obtained from Bruce N. Ames, Ph.D., while he was at the University of California-Berkeley; Berkeley, CA. The study followed the Principles of Good Laboratory Practices as formulated by the USFDA (1978).

REMARKS

Appropriate ethanol controls were included in the test. The bacterial auxotrophs utilized were: *S. typhimurium* TA 98, 100, 1535, 1537, and 1538. Neither *S. typhimurium* TA 102, nor *E. coli* strains WP2 *uvrA* nor WP2 (pKM101) were included. TA 1538 is not listed in the OECD Guideline 471 as an acceptable mutant for testing, but the other four used are listed. Since TNG is not an (classical) oxidizing agent, a cross-linking agent, nor a hydrazine, the absence of TA 102, or *E. coli* WP2 or WP2 (pKM101) should not have compromised the mutagenicity evaluation.

The S-9 metabolic fraction was prepared from the livers of Sprague-Dawley rats that had been pre-treated with Aroclor® (Registered Trade Mark of the Monsanto Co.) 1254 in their diet (sex, dietary level, duration of treatment, and post-dose interval before sacrifice were not reported). The S-9 mix also contained magnesium and potassium salts, glucose-6-phosphate, NADP, and phosphate buffer. Nine-tenths of a milliliter of S-9 mix (90µl of S-9 fraction) was added to each tube of top agar. The positive controls used are listed in OECD guideline 471, and included one control requiring activation and three that did not.

Standard contamination checks were performed concurrently. No contamination was found. The revertants detected in each assay were compared with the average background number and 95% confidence limits of revertants for each auxotroph found in over 60 previous trials for each auxotroph in the laboratory doing the testing. Revertant colonies were counted automatically. The data were analyzed manually. If the solvent control was within one S.D. of the historical mean and the highest increase in a test series was = 3X the solvent control value, the test material was considered mutagenic.

The evaluations of TNG with TA 1535 with S-9 and TA 100 with and without S-9, and the corresponding positive and negative controls for both auxotrophs had to be repeated. The repeats were necessary because the reversion frequency in TA 1535 plates without S-9 was outside the fiduciary limits and all plates with TA 100 obviously were contaminated. In this rerun with TA 1535, the previous three lowest doses were dropped and a new (higher) low dose plus two new higher intermediate doses were added to the tests with both auxotrophs.

The positive control requiring activation in the first set of replicates was 2-aminofluorene. It is not listed as an acceptable positive control in the 1997 version of OECD Guideline 471. The positive control requiring activation in the second set of replicates was 2-aminoanthracene. It is listed as a suitable control in that Guideline.

The test materials were plated in duplicate and all controls were plated in triplicate in both replicate sets of the evaluation.

The results of all assays are shown in the following table.

MATERIALS TESTED		MEAN SPONTANEOUS REVERTANTS/PLATE				
CONTROLS	S-9	TA 1535	TA 1537	TA1538	TA98	TA100
DMSO[1]	(-)	9	13	14	29	----
DMSO[1]	(+)	15	17	30	45	----

N-methyl-N'-nitro-N-nitrosoguanidine[2] (2.0µg/plate)	(-)	1152[5]	----	----	----	----
9-Aminoacridine[2] (150.0 µg / plate)	(-)	----	1209[5]	----	----	----
2-Aminofluorene[2] (10.0 (µg / plate)	(+)	----	----	1433[5]	1466[5]	----
NITROGLYCERIN(**µg / plate**)[3]						
5	(-)	9	14	15	27	----
16.6	(-)	8	14	17	29	----
50	(-)	9	12	14	33	----
166	(-)	17[5]	12	9	29	----
500	(-)	20[5]	8	8	25	----
5	(+)	19	22	31	42	----
16.6	(+)	18	20	28	39	----
50	(+)	19	20	29	35	----
166	(+)	25[5]	17	31	42	----
500	(+)	41[5]	14	27	38	----
CONTROLS						
DMSO[1]	(-)	-	----	----	----	133
DMSO[1]	(+)	12	----	----	----	122
N-methyl-N'-nitro-N-nitrosoguanidine[2] (2.0µg/plate)	(-)					1425[5]
2-Aminoanthracene[2] (2.5 µg / plate)	(+)	117[5]	----	----	----	949[5]
NITROGLYCERIN(µg / plate)[4]						
100	(-)	----	----	----	----	114
200	(-)	----	----	----	----	106
333	(-)	----	----	----	----	116
500	(-)	----	----	----	----	122
750	(-)	----	----	----	----	130
1000	(-)	----	----	----	----	100
100	(+)	16	----	----	----	116
200	(+)	17	----	----	----	94
333	(+)	31	----	----	----	103
500	(+)	35	----	----	----	121
750	(+)	54[5]	----	----	----	135
1000	(+)	68[5]	----	----	----	114

1. Positive Control.
2. Replicates 1 & 2 averaged.
3. Replicates 3 & 4 averaged.
4. Replicates 5 & 6 averaged.
5. Positive Response.

DATA QUALITY

Good. Study conducted at level of 1980 standards, when the "Ames Test" was first coming into use. Study was lacking in test improvements since that time. Results with TA 1535 agree with test in ethanol (See included Robust Summary) conforming to current standards.

REMARKS

This study lacked standard error or standard deviation values for the control background counts even though data from >60 previous assays was available at the laboratory. It is being included in this data set because of the irreproducible initial finding of reverse mutations in TA 1535 without S-9 and because Wink (Wink, *et al*., 1991), also reported that TNG was positive in TA 1535 without S-9 when the solvent was DMSO. The author of this Robust Summary has not conducted a literature survey for information on the effect of solvent on the "Ames Test".

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GENOTOXICITY ELEMENTS

15) *IN VITRO* MUTAGENICITY TO *SALMONELLA TYPHIMURIUM* MUTANTS

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occu-pational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

REMARKS

The material as received by the testing laboratory was a 10% (w/w) solution in absolute ethanol. The test solutions were prepared by diluting this stock solution with absolute ethanol.

METHOD

-

Although this test was carried out in 1986, the protocol tracked the bacterial reverse mutation assay as described in OECD Guideline No. 471 (OECD,1997), with one exception. The exception is described in the following **REMARKS** section. The plate incorporation method was followed. The starter cultures of the five auxotrophs used were obtained from Bruce N. Ames, Ph.D., while at the University of California-Berkeley; Berkeley, CA. The study followed the Principles of Good Laboratory Practices as formulated by the USEPA (1983), the USFDA (1980), and the OECD (1981). All doses and controls were run in triplicate.

REMARKS

Appropriate ethanol controls were included in the test. The bacterial auxotrophs utilized were: *S. typhimurium* TA 98, 100, 1535, 1537, and 1538. Neither *S. typhimurium* TA 102, nor *E. coli* strains WP2 *uvrA* nor WP2 (pKM101) were included. TA 1538 is not listed in the OECD Guideline 471 as an acceptable mutant for testing, but the other four used are listed. Since NG is not a classical oxidizing agent, a cross-linking agent, nor a hydrazine, the absence of TA 102, or E. coli WP2 or WP2 (pKM101) should not have compromised the mutagenicity evaluation.

The S-9 metabolic fraction was prepared from the livers of Sprague-Dawley rats that had been pre-treated with Aroclor® (Registered Trade Mark of the Monsanto Co.) 1254 in their diet. The S-9 mix contained 0.12 ml (44mg S-9 protein) of S-9 fraction / ml. It also contained magnesium and potassium salts, glucose-6-phosphate, NADP, and phosphate buffer. The sex of the rats and the duration and dosage of the feeding are not known. In the retest, the volume of the S-9 mix was slightly decreased and the amount of sterile distilled water was increased an equal volume. The respective volumes of S-9 mix used were: 120µ? and 100µ?. The positive controls selected are on the list in OECD guideline 471, and included a control requiring activation and three that did not.

Standard contamination checks were performed concurrently. No contamination was found. The revertants detected in each assay were compared with the average background number and 95% confidence limits of revertants for each auxotroph found in over 300 previous trials for each auxotroph in the laboratory doing the testing. Colonies were counted automatically and the counts were entered into the computer statistical program automatically. The statistical program of Moore and Felton (1983; a linear regression analysis) was used for analysis of the data.

The evaluations of TNG with 1)TA 1535 and S-9, 2)an ethanol control with S-9 and 3)the positive control 2-aminoanthracene with S-9, were repeated. In this rerun, the previous lowest dose was dropped and a new high dose was added. Cytotoxicity was seen at this new high dose, but there was a significant (95% C.L.) increase in reversions at the penultimate dose, the previous highest dose. The results of all assays are shown in the following table.

MATERIALS TESTED		MEAN SPONTANEOUS REVERTANTS/PLATE				
CONTROLS	S-9	TA 1535	TA 1537	TA1538	TA98	TA100
Ethanol	(-)	9 ± 6[a]	12 ± 4	11 ± 4	37 ± 7	111 ± 25
Ethanol	(+)	12 ± 4	12 ± 4	18 ± 2	27 ± 2	98 ± 12
Sodium Azide[1] (10 µg/plate)	(-)	373 ± 83[b]	----	----	----	377 ± 80[b]
9-Aminoacridine[1] (150 µg/plate)	(-)	----	693 ± 105[b]	----	----	----
2-Nitrofluorene[1] (5 µg/plate)	(-)	----	----	555 ± 28[b]	434 ± 62[b]	----
2-Aminoanthracene[1] (2.5 (µg/plate)	(+)	152 ± 16[b]	158 ± 10[b]	1059 ± 160[b]	1040 ± 178[b]	762 ± 86[b]
NITROGLYCERIN(**µg/plate**)[2]						
15	(-)	8 ± 6	10 ± 4	10 ± 6	48 ± 14	118 ± 27
50	(-)	5 ± 4	9 ± 6	10 ± 5	43 ± 6	67 ± 7
150	(-)	6 ± 3	6 ± 2	6 ± 3	35 ± 8	108 ± 24
500	(-)	12 ± 5	7 ± 2	11 ± 4	38 ± 5	89 ± 35
1500	(-)	4 ± 2	5 ± 1	7 ± 5	36 ± 7	7 ± 5
15	(+)	14 ± 10	11 ± 5	18 ± 7	39 ± 6	84 ± 3
50	(+)	12 ± 2	13 ± 8	25 ± 2	32 ± 6	84 ± 9
150	(+)	12 ± 8	11 ± 3	18 ± 4	38 ± 10	88 ± 11
500	(+)	26 ± 8[b]	16 ± 4	16 ± 1	36 ± 6	121 ± 12
1500	(+)	31 ± 5[b]	8 ± 4	9 ± 3	33 ± 8	94 ± 23
(Table continued on next page)						
CONTROLS[3]						
Ethanol	(-)	----	----	----	----	----

Ethanol	(+)	18 ± 7	----	----	----	----
2-Aminoanthracene[1] (2.5 µg/plate)	(+)	203 ± 7[b]	----	----	----	----
NITROGLYCERIN(µg/plate)[3]						
50	(+)	20 ± 5	----	----	----	----
200	(+)	20 ± 8	----	----	----	----
500	(+)	27 ± 4	----	----	----	----
1500	(+)	51 ± 2[b]	----	----	----	----
2000	(+)	41 ± 6[b]	----	----	----	----

1. Positive Control
2. Trials 1 & 2 averaged
3. Trial 3

a. Standard Deviation
b. Positive Response

DATA QUALITY

Excellent. This is a **KEY STUDY**

REMARKS

A similar spectrum of activity has been reported by scientists at the U. S. National Cancer Institute (Maragos, C.M., *et al*., 1993) and by scientists on a U.S. Army project (Ellis, *et al*., 1978). [A robust summary of the latter is included in this set of summaries (Comment of author of Robust Summary)].

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GENOTOXICITY ELEMENTS
15) *IN VITRO* MUTAGENICITY TO *SALMONELLA TYPHIMURIUM* MUTANTS

TEST SUBSTANCE – IDENTITY/PURITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occu-pational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

The test material was received by the testing laboratory as a "10% mixture in lactose" (9.78% by elemental analysis and gas chromatography) (Ellis, *et al*., 1978, Lee, *et al*., 1975). However, the test report (Ellis, *et al*., 1978) does not say whether the TNG was extracted from the lactose for this mutagenicity test and evaluated as the neat material. In all other toxicology tests reported in this series of acute tests (Lee, *et al*., 1975), the test material was the lactose / TNG complex admixed with other adjuvants. Therefore, it must be assumed that the actual test material here was TNG adsorbed on lactose (9.78% a.i.) and that the concentrations reported are the actual concentrations of TNG, as were reported for other tests reported in Lee, *et al*., 1975.

REMARKS

The test solutions of TNG were prepared by suspending this stock material in DMSO. It was not filtered after suspending the lactose / TNG test material.

METHOD

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This test was carried out in 1975. The protocol was the original bacterial reverse mutation assay as described by Dr. Ames (Ames, 1975). It contained many of the elements of OECD Guideline No. 471 as issued on 21 July, 1997 (OECD, 1997), with the exceptions described in the following **REMARKS** section. The plate incorporation method was followed. The starter cultures of the five auxotrophs used were obtained from Bruce N. Ames, Ph.D., while he was at the University of California-Berkeley; Berkeley, CA.

REMARKS

This study pre-dated the first GLPs by five years. It has deficiencies, but is being reported in this SIDS package because it reports the reverse mutational activity of TNG against *S. typhimurium* mutant TA 1537 in the absence of rat liver S-9. This was not observed in the other two reverse mutation tests with TNG being reported.

The bacterial auxotrophs utilized were: *S. typhimurium* TA 98, 100, 1535, 1537, and 1538. Neither *S. typhimurium* TA 102, nor *E. coli* strains WP2 *uvrA* nor WP2 (pKM101) were included. Since TNG is not a classical oxidizing agent, a cross-linking agent, nor a hydrazine, the absence of TA 102, or E. coli WP2 or WP2 (pKM101) should not have compromised the mutagenicity evaluation.

The S-9 metabolic fraction was prepared from the livers of male Charles River CD rats that had been induced only with sodium phenobarbital (daily i.p. doses of 80gm / kg b.wt. daily for four consecutive days). Twenty-four hours after the last injection the livers were removed and the S-9 was prepared. It also contained magnesium and potassium salts, glucose-6-phosphate, NADP, and phosphate buffer. The dose of S-9 / culture was not reported.

The positive controls and their doses were: 7,12 dimethyl-benzanthracene (20 µg/plate), benzo[a]pyrene (5 µg/plate), and cyclophosphamide (200 µg/plate). The results with the positive and solvent controls were not reported. Individual plate counts were not reported. Only the ratios of the average numbers of reversions in the test plates divided by the average numbers of reversions in the control plates were reported. A test was considered positive if this ratio =2.0. If the ratio was = 2.0 at a concentration <100 µg/plate, the chemical was considered a "strong" mutagen; if >100 µg/plate, it was considered a "weak" mutagen. Each test and control plate was run in duplicate. Revertant colonies were counted manually. The data were analyzed manually.

RESULTS

NITROGLYCERIN (µg/plate)		REVERTANT RATIOS **(TEST/CONTROL)**				
	S-9	TA 1535	TA 1537	TA1538	TA98	TA100
10	(-)	1.0	0.8	1.0	1.2	1.4
100	(-)	0.9	0.9	1.0	1.0	0.9
300	(-)	0.8	1.0	N.D.[2]	1.5	1.0
1,000	(-)	0.8	4.0[1]	N.D.	0.6	0.8
10	(+)	1.0	1.2	0.4	1.0	0.7
100	(+)	1.0	0.8	0.8	0.9	0.7
300	(+)	1.8	1.0	0.4	0.9	1.0
1,000	(+)	4.0[1]	0.5	0.2	0.6	0.7

1. Significant increase in ratio.
2. No data.

CONCLUSIONS

The test is not up to OECD 1997 standards, but the results reported for TA 1535 in the presence and absence of S-9 are consistent with the results presented in the accompanying reports, in this SIDS package, for TNG tested in DMSO and in ethanol and using more contemporary methodology. Therefore, the results with TA 1537 must be considered seriously. If so, why wasn't a corresponding increase in TA1537 seen in the reverse mutation studies with TNG in ethanol and in DMSO? The answer could lie in the dosages selected in the three reports. The highest dose in the accompanying reverse mutation test report of TNG in DMSO was 500µg / plate, where no effect was seen in TA 1537. In the accompanying reverse mutation test using ethanol as a solvent, also no effect was seen in TA 1537 at 500µg/plate. There was no test at 1,000µg / plate in that study, and there appears to have been some toxicity at 1500 µg / plate. So the increased number of reversions seen in the instant test at 1000µg / plate in the absence of S-9 must be considered real. Submitter has not done a literature survey for mutagenicity studies on lactose. (Conclusions and comments those of author of this Robust Summary).

DATA QUALITY

Acceptable. Study conducted at level of 1978 standards, when the Ames Reverse Mutation test was first coming into use. Study was lacking in test improvements since that time. Results with TA 1535 agree with tests conforming to current standards. Results with TA 1537 are new, but appear to be valid.

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SIDS_TNG_Ames1975DMSOFinal

HUMAN HEALTH EFFECTS

16) REPEATED DOSE TOXICITY

ONE YEAR ORAL (CAPSULE) DOSING IN DOGS

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These all are cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

Production grade TNG adsorbed on lactose to give a product analyzing for 9.72 ± 0.09% (w/w) TNG was used in the capsules with which the dogs were dosed once daily. It was analyzed by GC (flame ionization detector). No peaks other than those from TNG were seen, with a detection limit (*vs*. TNG = 100%) of 1 % for other components. It also was analyzed for TNG *per se* by the method of Wells (1970). In addition, a sample of the TNG / lactose test material was extracted with chloroform and an aliquot of the extract was evaporated to yield a residue whose infrared spectrum (between salt plates) was determined. It was identical to that reported for TNG (Hayden, *et al*., (1972).

METHOD

METHOD FOLLOWED: Method corresponded to OECD Guideline #452 (Adopted 1981), except as noted below.
TEST TYPE: Daily oral administration of one or two gelatin capsules.
GLP**: Unlikely. Study pre-dated even USFDA GLPs.**
YEAR: 1976-1978.
SPECIES: Beagle; young adults from Hazleton Research Animals, Cumberland, Virginia.
SEX: Male & female.
ROUTE of ADMINISTRATION: Oral (daily).
DURATION: Twelve months dosing and one month recovery.

DOSE LEVELS: 0.0, 1, 5, and 25 mg TNG / kg / day. Amounts of test material required for the doses were adjusted weekly, based on the weekly weight of each dog. The dogs receiving the 5 mg / kg dose received one capsule daily, containing the appropriate amount of the 9.72 % concentration of TNG on lactose. Dogs receiving the 25 mg / kg dose received half the appropriate amount of the same formulation in each of two sequentiallly administered capsules. For the dogs receiving the 1 mg / kg / dose, the TNG / lactose mixture was further diluted with lactose to 2% TNG and they were given their daily dose in one capsule containing this diluted TNG / lactose. Control animals received daily doses of lactose equal to that received by the high dose dogs, also from two sequential capsules. All animals were dosed seven days a week.

EXPOSURE PERIOD: Twenty-four hours / day (continuous) for one year.

POST-EXPOSURE PERIOD: One month.

STATISTICAL METHODS: In general, standard methods (Steel & Torrie, 1960) with $p<0.05$ considered significant. Continuous variables were analyzed by Dunnett's multiple comparison procedure after an analysis of variance, or Student's *t* test. Enumeration data, such as tumor incidence, were analyzed by Fisher's exact probability test. In some of the histopathological incidence analyses the CHI square test or exact probabilities on contingency tables were used with $p<0.05$ considered significant.

REMARKS

DEVIATIONS FROM OECD PROTOCOL #452. Solubility and hydrolysis character-istics, f.pt., and m.pt. of NG were not determined for this study. They are published (see appropriate included Robust Summaries). Blood (jugular) clinical chemistry and hematology analyses were performed at zero, three, six, nine, and twelve months. Three males and three females were sacrificed from each group after twelve months. There was a one month recovery period for the remaining three males and three females in each group. Total protein and albumin were not determined in the blood samples. There were no urinalyses. Rectum, femur, and aorta were not routinely examined microscopically. In addition to the organs called for in OECD Protocol #452, diaphragm, tongue, tonsils, trachea, and ureter routinely were removed, fixed, and examined microscopically.

At the end of the recovery period, blood samples were taken from the recovery dogs for selected hematology measurements. The recovery dogs were then sacrificed and organs were removed, processed, sectioned, and examined microscopically as described above.

RESULTS

NOAEL**. Under the conditions of this study, there was no NOAEL in either sex.**

LOAEL. Under the conditions of this study, the LOAEL in both sexes was the lowest level tested, 1 mg TNG / kg / day.

REMARKS / DISCUSSION.

As in most studies where blood chemistries and hematology are determined, there were random, one-time, statistically significant, excursions above or below the control animal values for that component and at all other times the values were within normal ranges. These excursions were felt to have no toxicological importance. Methemoglobin, on the other hand, while also having only one statistically significant elevation (Dunnett's multiple comparisons procedure) also had a more or less consistent time-related dose-response relationship, especially at nine and 12 months, and after one month recovery, as shown in Table 1, and was not a usual blood component, that would be subject to daily variations. Except for the high-dose males, blood levels of methemoglobin returned to zero by the end of the one-month recovery period, as also shown in Table 1.

TABLE 1

		BLOOD METHEMOGLOBIN CONCENTRATIONS (%)					
DOSE (mg NG/kg/day)	SEX	MONTHS ON TEST					RECOVERY
		0	3	6	9	12	
0	M	0	0	1.1[1]	0	0	0
	F	0	0	0.3	0.5	0	0

1	M	0	0	0.5	0.9	0.2	0
	F	0	0	0	0	0.2	0
5	M	0	0	0.9	0	0.5	0
	F	0	0	0.2	0.5	0.4	0
25	M	0	0	1.5	1.1[2]	0.9	1.0
	F	0	0	0.2	1.6	0.8	0

1. Uncertainties in numbers not shown.
2. Significant elevation (Dunnett's multiple comparison procedure).

The elevated levels of methemoglobin were not accompanied by any of the usual *sequelae* of a severe methemoglobinemia, *e.g.,* Heinz bodies, elevated reticulocyte counts, and anemia.

There were no consistent dose-response relationships in food consumption or weight gains in either males or females.

CONCLUSIONS

The only effect that could be attributed to TNG in this study was a mild methemo-globinemia that was not accompanied by any of the *sequelae* of a severe methemoglobinemia such as Heinz bodies, elevated reticulocytes, and anemia. Except for the dogs receiving 25 mg TNG / kg / day, methemoglobin levels returned to zero after a thirty day recovery period.

QUALITY

Excellent. This was a well designed study, based on the generally accepted sequence of toxicological studies on a chemical. This is a **KEY STUDY**.

REMARKS

The design of this study was based on a previous 13-week (90 day) study in which no effects were seen in dogs daily receiving encapsulated oral doses of the same NG formulation. Those dogs initially received daily dosages of 0, 0.01, 0.1, and 1.0 mg NG / kg / day for four successive weeks. One male and one female were then euthanized after removing blood for chemical and hematologic evaluations. Organs of this male and female were then removed for histopathologic examination. One male and one female also were put on "recovery" status. The remaining animals then received encapsulated doses of 0, 0.05, 0.5 and 5.0 mg NG daily for nine successive weeks. Half of the animals of each sex in each group were then euthanized and the same toxicity evaluations were carried out as at four weeks and in the instant one-year study. The remainders were put on recovery for four weeks. They then were euthanized and subjected to the same toxicological studies as those sacrificed after four weeks. No statistically significant toxicological effects of any sort were seen in any of those animals. As the instant study shows, no effects were to be expected by 13 weeks, the usual length of the ultimate study preceding a two-year study.

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SIDS_TNG_OralDog1yrCapsulFinal

HUMAN HEALTH EFFECTS
16 REPEATED DOSE TOXICITY
TWO YEAR DIETARY STUDY IN RATS

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for nitroglycerin (TNG) in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

Pure TNG was used to prepare a stock diet concentrate from feed dried to 0.1 % moisture. The concentrate was analyzed by GC (flame ionization detector). No peaks other than TNG were seen, with a detection limit (*vs*. TNG = 100%) of 1 % for other components. It also was analyzed for TNG *per se* by the method of Wells (1970). (This information was used to guide the weekly preparation of the diets.) In addition, a sample of the diet was extracted with chloroform and an aliquot of the extract was evaporated to yield a residue whose infrared spectrum (between salt plates) was determined. It was identical to that reported for TNG (Hayden, *et al*., (1972).

The diet concentrate was stored in plastic bags which were then stored in five gallon glass jars sealed by wooden plugs covered with aluminum foil. The plugs were held in place by springs. The jars were stored in an underground magazine. Test levels of diet were prepared fresh weekly by dilution of the concentrate with unheated feed and were analyzed after preparation. Control analyses were carried out to measure evaporation of NG from the diet under cage conditions during the week. GC also was used for these analyses, but with a ^{63}Ni detector. This information was used to calculate actual dosage received by the rats. Feeders were topped off with fresh diet on the fourth day of each week, and diet in each feeder was replaced totally every seven days. The feed for the control rats contained 10 % (w/w) of diet that had been dried to 0.1 % water content.

METHOD

METHOD FOLLOWED: Method corresponded to OECD Guideline #452 (Adopted 1981), except as noted below.
TEST TYPE: Dietary administration for two years.

GLP**: No. Study pre-dated even USFDA GLPs.**
YEAR: 1976-1978.
SPECIES: Albino rat.
STRAIN: Charles River CD.
SEX: Male & female.

ROUTE of ADMINISTRATION: Oral (dietary).

DOSE LEVELS: 0.0, 0.01, 0.1 1.0% (w/w) of diet. Corresponds to male and female daily intakes of 0.0, 3.04 ± 0.16, 31.5 ± 1.6, 363 ± 10 and 3.99 ±0.18, 38.1 ±1.6, and 434 ± 11 mg NG / kg day, respectively (means ± standard errors of 24 monthly measurements).

EXPOSURE PERIOD: Twenty-four hours / day (continuous) for two years.

STATISTICAL METHODS: In general, standard methods (Steel & Torrie, 1960), with $p<0.05$ considered significant. Continuous variables were analyzed by Dunnett's multiple comparison procedure after an analysis of variance or Student's *t* test. Enumeration data, such as tumor incidence, were analyzed by Fisher's exact probability test. In some of the histopathologic incidence analyses the CHI square test or exact probabilities on contingency tables, with $p<0.05$ considered significant.

REMARKS

DEVIATIONS FROM OECD PROTOCOL #452. Solubility and hydrolysis character-istics, f.pt., and m.pt. of TNG were not determined for this study. They are published (see appropriate included Robust Summaries). Food intake was measured weekly for the first four weeks and for one week / month, thereafter. Body weights were measured weekly until the body weight leveled off, and biweekly thereafter. Blood was collected for hematology analyses from tail tips of (where possible) the same four males and four females before the test started, and at the end of 3, 6, 9, 12, 18, and 24 months. Interim blood chemistry analyses were performed at twelve months. There were no urinalyses. Rectum, femur, and aorta were not routinely examined microscopically. Femurs from these rats were, however, used as a source of bone marrow cells for the Rat Bone Marrow Chromosome Aberration study reported in the accompanying Robust Summary of that title.

At the end of 12 months and 24 months, four males and four females from each group were taken off the test diet, put on "control" diets, and maintained as recovery groups, under conditions otherwise identical to those of the test and control groups. These two sets of "recovery" rats were sacrificed at the end of months 13 and 25, respectively, and subjected to the same clinical, hematological, macroscopic, and microscopic examinations as the animals sacrificed at 12 and 24 months, those sacrificed *in extremis*, and the unscheduled deaths. Also, after twelve months on test, abdominal aortal blood was taken for hematology and clinical chemistry from four males and four females from each group. These animals were then sacrificed, necropsied, and their organs examined for histopathologic effects.

In addition, four males and four females from each group were scheduled for sacrifice at each of 3, 6, 9, and 18 months for the same 1)blood hematologic and chemical, 2)gross whole-body and organ, and 3)histopathologic examinations. Any remaining survivors at 24 months were scheduled for the same examinations.

PROTOCOL OVERVIEW. Thirty-eight males and 38 females were used per group. Except as given above, unscheduled deaths, and any animals euthanised *in extremis*, they were sacrificed after 24 months on test. Animals were examined daily for clinical signs. At termination, blood was collected from the abdominal aortae of the remaining rats following ether anesthesia. Animals dying spontaneously were examined externally, and their internal organs were closely examined macroscopically. Whenever scientifically reasonable, and at all scheduled sacrifices, the usual internal and external organs were evaluated grossly, weighed, and then processed for microscopic evaluation. Organ / body weight ratios were calculated.

HEMATOLOGY. This battery included: erythrocyte, reticulocyte, leucocyte, and platelet counts; hematocrit, hemoglobin, erythrocyte indices, methemoglobin, Heinz bodies, and clotting time.

CLINICAL CHEMISTRY. This battery included: fasting blood glucose, serum SGOT, serum SGPT, APase, and BUN. Special tests, *e.g.*, serum electrolytes were to be performed whenever indicated

ORGAN AND OTHER WEIGHTS. Brain, heart, liver, kidneys, spleen, and gonads.

RESULTS

NOAEL. The low dose (24 month ave. in males = 3.04 ± 0.16 mg TNG / kg /day; in females = 3.99 ± 0.18 mg TNG / kg / day) was a "no-effect" dose in males and females by any of the criteria used.

LOAEL. The middle dose (24 month ave. in males = 31.5 ± 1.6 mg TNG / kg / day; in females = 38.1 ± 1.6 mg TNG / kg / day) was an effect dose in both males and females.

EFFECTS

ALL DOSES

INCIDENCE (%) OF RELEVANT HISTOPATHOLOGIC LESIONS BY GROUP AND SEX AT 24 MONTHS

DOSE (% IN FEED)	0		0.01		0.10		1.0	
SEX	Male	Female	Male	Female	Male	Female	Male	Female
LESION								
PITUITARY Chromophobe adenoma	6	79	48	66	56	78	26	29
LIVER								
Cholangiofibrosis	0	0	0	0	0	0	86	96
Cystic bile duct hyperplasia	0	0	0	0	0	0	29	72
Adenomatoid bile duct hyperplasia	0	0	0	0	0	0	24	24
Areas/foci of hepatocellular alteration	25	24	50	25	65	71	51	80
Neoplastic nodules	4	0	0	3	4	4	10	20
Hepatocellular carcinoma	0	0	0	0	12	7	62	44
SPLEEN: Hyperpigmentation	13	3	7	6	23	18	29	64
TESTIS: Interstitial cell tumor	8	--	4	--	12	--	52	--
KIDNEY: Epithelial hyperpigmentation	4	0	0	9	0	11	29	84
MAMMARY GLAND								
Tumor (all types)	4	45	0	59	4	43	0	8
Fibroadenoma	100[1]	85	0	74	100	67	0	100
Adenoma	0	46	0	16	0	42	0	0
Fibroma	0	0	0	0	0	17	0	0
Adenomacarcinoma-carcinoma	0	8	0	11	0	17	0	0

1. Per cent of tumors found that fell in this class. Obviously, some rats had more than one type of mammary tumor (Comment of author of Robust Summary).

HIGH DOSE

CLINICAL / LABORATORY / HEMATOLOGICAL TOXIC EFFECTS ATTRIBUTABLE TO TNG @ 1% DIETARY LEVEL[1]								
EFFECT	TEST MONTH							
	3	6	9	12	12+ 1	18	24	24 + 1
Body Weight (% of Controls)* F	71	74	85	85	78	74	77	88
	77	76	74	66	71	62	57	66
NG Intake M (mg/kg/day) F	390	370	315	370	0	350	363[2]	0
	420	440	335	470	0	430	434[2]	0
Food Consumption (% of Controls)							89[2]	
							78[2,3]	

Liver Weight M (% of controls) F				153[4]	142[4]		359[4]	286[4]
				150[4]	171[4]		210[4]	287[4]
Liver Wt. / Body Wt.[5] (% of controls)				175	182		475	308
				182	139		368	437
Glucose, fasting (mg %)							M[3]	
SGOT (IU/ L)							M[4]	
SGPT (IU/ L)							M[4]	
BUN (mg %)							F[4]	
Alk. Phosphatase (IU/ L)							M[4]	
Methemoglobin (%)	M[4], F[4]	M[4], F[4]	M[4]	M[4], F[4]		M[4], F[4]		
Hematocrit (vol %)	M[4], F[4]							
Hemoglobin (gm %)	M[4], F[4]							F[3]
Reticulocytes (%)	M[4]		M[4]	M[4]			F[4]	
Erythrocytes (x 10^6 / mm^3)	F[4]	F[4]						

* Initial group average body weights were not reported. It is assumed by the author of this Robust Summary that all were essentially equal.

1. None of these or other effects had a significant incidence at lower dose levels, except hemoglobin concentration (See "Middle Dose" below).
2. Twenty-four month average.
3. Significantly decreased by Dunnett's multiple comparison procedures.
4. Significantly elevated by Dunnett's multiple comparison procedures.
5. Calculation by author of Robust Summary. No statistical evaluation made.

As shown in the table in the above "All Doses" Section, the incidence of 1) pituitary chromophobe adenomas in males and females and 2) mammary tumors of all types were dramatically reduced in females at the high dose. The authors did not report the statistical significance, if any, of this reduction.

MIDDLE DOSE: Produced substantial increases in areas/foci of hepatocellular alteration, hepatocellular carcinomas (see Table above). It also caused increases in splenic pigmentation. Hemoglobin was significantly (Dunnet's multiple comparison procedures) reduced in a dose-related fashion in females at the end of the one month recovery period following twenty-four months of dietary administration.

There were several isolated instances of statistically significant (Dunnett's multiple comparison procedures) increases or decreases of various clinical or hematological measurements at this dose level. However, there was never a corresponding statistically significant increase or decrease in these same measurements at the high dose. The investigators did not include them as effects at the middle dose, and the author of this Robust Summary agrees that these were isolated aberrations such as frequently occur with these measurements and were of no toxicological significance. Regardless, the investigators had already classified the middle dose as an "Effect Dose" based on serious carcinogenic effects. (Comment of author of Robust Summary)

LOW DOSE: As at the middle dose, there were occasional isolated instances of statistically significant increases or decreases of various clinical or hematological measurements at this dose level (Dunnett's multiple comparison procedures). However, there was never a dose-response or time-continuum relationship. The investigators did not consider them to be effects at the low dose, and the author of this Robust Summary agrees that these were isolated aberrations such as frequently occur with these measurements and were of no toxicological significance.

SURVIVAL: Control males reached the 50% mortality level at 21 ½ months; high dose males at 22 ½ months. Control females reached 50% mortality at 23 months; high dose females had only 30% mortality at 24 months. Control females had a 30% mortality at 20 months.

TUMOR TD_{50} VALUES: **TD_{50} values for the rat tumors were calculated by Gold & Zeiger(1997). The values are:**

TUMOR	TD_{50}	Two-tailed p Value
Female Mixed Liver	329 mg / kg	p<0.0005
Male Mixed Liver	**221 mg / kg**	**p<0.0005**

Male Testicular Interstitial Cell	**405 mg / kg**	**$p<0.0005$**

DISCUSSION

At the end of the one month recovery period following twenty-four months of oral ingestion, there was a statistically significant (Dunnett's multiple comparison procedures), but not dose-related, decrease in hematocrit in the female rats receiving test material at all three dose levels. The value reported was the same (within experimental error) for the females at all three test levels. There was no significant change in mean corpuscular volume, or erythrocyte count. In addition, at the end of the 24 month feeding period, the low dose rats had a composite hematocrit value that was exactly the same as the control rats and the high dose rat groups' composite hematocrit was 5% higher than the controls. Throughout the 24 month test period, there was never a significant decrease or increase in hematocrit in either males or females, at any of the test levels (measurements at 0, 3, 6, 9, 12, 18, and 24 months). The investigators considered the low dose level to be a "No Effect" level (Ellis, *et al*., 1978 and Ellis, *et al*., 1984) and the author of this Robust Summary agrees that this apparent decrease in hematocrit simply reflects either an unfortunate alliance of the gods of variance or a technical glitch in the measurement or recording procedures and that the low-dose level was a "No-Effect" level.

The high dose females had a dramatic decrease in the terminal incidence of pituitary chromophobe adenomas and mammary gland tumors in addition to the decrease in deaths mentioned above. In fact, the mortality curve for the high dose group of females crossed under the control line at 18½ months. The investigators speculated that this longevity increase in the females at the high dose may have been due to the decreased tumor incidence.

DATA QUALITY

Excellent. Comprehensive study. Lacked some features recommended in the OECD Guideline 452 (published after the study was completed), but included some features that are not in this OECD Guideline. This is a **KEY STUDY**.

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HUMAN HEALTH EFFECTS

16 REPEATED DOSE TOXICITY

TWO YEAR DIETARY STUDY IN MICE

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These are all cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

Pure TNG was used to prepare a stock diet concentrate from feed dried to 0.1 % moisture. The concentrate was analyzed by GC (flame ionization detector). No peaks other than TNG were seen, with a detection limit (*vs*. TNG = 100%) of 1 % for other components. It also was analyzed for TNG *per se* by the method of Wells (1970). (This information was used to guide the weekly preparation of the diets.) In addition, a sample of the diet was extracted with chloroform and an aliquot of the extract was evaporated to yield a residue whose infrared spectrum (between salt plates) was determined. It was identical to that reported for TNG (Hayden, *et al*., (1972).

The diet concentrate was stored in plastic bags which were then stored in five gallon glass jars sealed by wooden plugs covered with aluminum foil. The plugs were held in place by springs. The jars were stored in an underground magazine. Test levels of diet were prepared fresh weekly by dilution of the concentrate with unheated feed and were analyzed after preparation. Control analyses were carried out to measure evaporation of TNG from the diet under cage conditions during the week. GC also was used for these analyses, but with a ^{63}Ni detector. This information was used to calculate actual dosage received by the rats. Feeders were topped off with fresh diet on the fourth day of each week, and diet in each feeder was replaced totally every seven days. The feed for the control rats contained 10 % (w/w) of diet that had been dried to 0.1 % water content.

METHOD

METHOD FOLLOWED: Method corresponded to OECD Guideline #452 (Adopted 1981), except as noted below.
TEST TYPE: Dietary administration for two years.

GLP**: No. Study pre-dated even USFDA GLPs.**
YEAR: 1976-1978.
SPECIES: Albino Swiss mouse.
STRAIN: Charles River CD-1.
SEX: Male & female.
ROUTE of ADMINISTRATION: Oral (dietary).

DOSE LEVELS: 0.0, 0.01, 0.1, and 1.0% (w/w) of diet. Corresponded to male and female daily intakes of 0.0, 11.10 ± 0.40, 114.6 ± 4.6, 1022 ± 38 and 0.0, 9.72 ± 0.29, 96.4 ± 3.3, and 1058 ± 31 mg TNG / kg / day, respectively (means ± standard errors of 24 monthly measurements, except the male high dose, which was only 23 measurements because of unexpected deaths).

EXPOSURE PERIOD: Twenty-four hours / day (continuous) for two years.

STATISTICAL METHODS: In general, standard methods (Steel & Torrie, 1960) with $p<0.05$ considered significant. Continuous variables were analyzed by Dunnett's multiple comparison procedure, after an analysis of variance or Student's *t* test. Enumeration data, such as tumor incidence, were analyzed by Fisher's exact probability test. In some of the histopathological incidence analyses the CHI square test or exact probabilities on contingency tables were used with $p<0.05$ considered significant.

REMARKS

DEVIATIONS FROM OECD PROTOCOL #452. Solubility and hydrolysis character-istics, f.pt., and m.pt. of NG were not determined for this study. They are published (see appropriate included Robust Summaries). Food intake was measured weekly for the first four weeks and for one week / month, thereafter. Body weights were measured weekly until the body weight leveled off, and biweekly thereafter. Interim blood clinical chemistry and hematology analyses were performed at twelve months. There were no urinalyses. Rectum, femur, and aorta were not routinely examined microscopically.

At the end of 12 months and 24 months, four males and four females from each group were scheduled to be put on "control" diets and maintained as recovery groups, under conditions otherwise identical to those of the test and control groups. These "recovery" rats were to be sacrificed at the end of months 13 and 25, respectively, and subjected to the same clinical, hematological, macroscopic, and microscopic examinations as the animals at 12 and 24 months, those sacrificed *in extremis*, and the unscheduled deaths. Also, after twelve months on test, abdominal aortal blood was taken for hematology and clinical chemistry from four males and four females from each group. These animals were then sacrificed, necropsied, and their organs examined for histopathologic effects.

PROTOCOL OVERVIEW. Fifty-eight males and 58 females were used per group. Except as mentioned above, they were sacrificed after 24 months on test. Animals were examined daily for clinical signs. At termination of the study, blood was collected from the abdominal aortae of the remaining mice following ether anesthesia. Animals dying spontaneously were examined externally, and their internal organs were closely examined macroscopically. Whenever scientifically reasonable, and at all scheduled sacrifices, the usual internal and external organs were evaluated grossly, weighed, and then processed for histopathologic evaluation. Organ / body weight ratios were calculated.

HEMATOLOGY. This battery included: erythrocyte, reticulocyte, leucocyte, and platelet counts; hematocrit, hemoglobin, erythrocyte indices, methemoglobin, Heinz bodies, and clotting time.

CLINICAL CHEMISTRY. This battery included: fasting blood glucose, serum SGOT, serum SGPT, APase, and BUN.

ORGAN WEIGHTS. Brain, heart, liver, kidneys, spleen, and gonads.

RESULTS

NOAEL. The middle doses (24 month averages in males = 114.6 ± 4.6 mg TNG / kg /day and 96.4 ± 3.3 mg TNG / kg / day in females were considered "non-toxic" doses by the investigators.

LOAEL**. The high doses (24 month averages in males = 1022 ± 38mg TNG / kg /day and 96.4 ± 3.3 mg TNG / kg / day in females) were considered "toxic" doses by the investigators.**

TOXIC EFFECTS. Except for the higher death rate in the high dose males, and despite the higher daily intake of TNG (in mg TNG / kg of mouse) at each dietary level of TNG in the mouse study, the toxic effects in the mice were not nearly as dramatic as in the rats.

The investigators felt that the middle dose was the NOAEL, and that the high dose was the LOAEL based on a combination of observations: Lower feed consumption and weight gain, behavioral effects (unspecified), and methemoglobinemia. Also present were *sequelae* of methemoglobinemia such as Heinz bodies, compensated anemia, and pigment deposits. However, many of these same factors also were elevated at the middle dose level and hepatic pigment deposits were seen even at the low dose in some of the mice dying off-schedule during the second year (Observations of the author of this Robust Summary).

For example, methemoglobin levels in the males were significantly (Dunnett's multiple comparisons procedures) elevated in the middle dose males at 12 months to almost the same level as in the high dose. The methemoglobin was not elevated in the females at the middle dose, but it was at the high dose. Heinz bodies were significantly elevated (Dunnett's multiple comparisons procedures) in the high dose males and females at twelve months. They were insignificantly elevated in the mid-dose females and not elevated in the mid-dose males. They were elevated in the middle and high dose females at 24 months, but the elevation was significant (Dunnett's multiple comparisons procedures) only in the high dose females. The per cent reticulocytes was elevated in both the mid-dose and high dose females in the 24 month groups, but the elevation was statistically significant (Dunnett's multiple comparison procedures) only in the middle dose females (Observations of the author of this Robust Summary).

The two consistent effects that the investigators felt were due to TNG treatment were 1) deposition of a granular, golden-brown pigment in various organs and discrete cells and 2) excessive hepatocellular dysplasia.

The golden-brown pigment gave only a weak Prussian blue reaction, so the investigators did not think it was hemosiderin. They did not try to characterize it any further. The incidence is shown in the following table.

INCIDENCE OF PIGMENT DEPOSITS								
DOSE (% in Diet)	0		0.01		0.1		1.0	
SEX	Male	Female	Male	Female	Male	Female	Male	Female
TIME & ORGAN / CELL								
12 Months								
Liver							4/4	2/4
12 + 1 Month Recovery								
Liver							4/4	2/4
Spleen							1 / 4	4/4
24 Months							--[1]	
Liver						4/8	--	5/6
Spleen							--	2/6
Adrenal							--	1/6
Ovary[2]						2/8	--	1/6
Macrophages					2/2		--	
24 + 1 Month Recovery							--[1]	
Liver							--	2/2
Ovary[2]							--	1/2
Unscheduled Deaths								
26 – 52 Weeks								
Liver							2/2	
53 – 104 Weeks								
Liver			2/18	1/11		1/8	1/5	1/7
Spleen					1/8	2/8	5/5	5/7
Kidney							2/5	3/7
Thyroid								1/7
Lymph Node	1/9							

1. No male survivors in the 24 month group.
2. Stromal cells only.

When one examines the histopathologic information for the unscheduled deaths, it can be seen that the discernible pigment deposition even occurs at the 0.01 % dose level, and in one control. But since it seems to have a dose-related spectrum of organ incidence, it probably is associated with the NG administration (opinion of the investigators).

An hepatocellular "dysplasia" also was observed in the histopathologic exami-nations. It apparently was different from the areas / foci of hepatocellular "alteration" seen in the rats. It was seen in test and control males and females. It ranged in severity from minimal / equivocal to "marked". The investigators considered its presence at minimal / equivocal severity to be background "noise". They considered higher levels of severity to be test-material-related. It was credibly found at these higher levels of severity only in the males and only at their 12 month and 12 + 1 month (recovery) sacrifices. At both sacrifices it was present at this severity in 100 % (4/4) of the high dose males, and at lesser percentages in the lower doses and controls. It was present at this severity in 1 / 3 Control males at the 12 + 1 month sacrifice. Because of the small number of rats involved at each dose level (3-4), it hard to say there was a dose-response relationship, although one was apparent at the 12 month sacrifice. (Observation of the Robust Summary author. However, the author of this robust summary also believes this was just fortuitous).

The females at the 12 and 12 +1 month sacrifices had the same percentage of rats affected and at the same degree of severity (minimal / equivocal) in both the control group and the high dose group. The lesion was not seen in the low and middle dose groups at either sacrifice. At the 24 and 24 + 1 month sacrifices, it was seen in all groups, including controls, at essentially the same frequency and only at the background severity level (minimal / equivocal).

In the unscheduled deaths during the 1-12 month and 13-24 month periods, it again was seen at essentially Control Group frequencies and severity in all groups, males and females.

Mice fed the high dose lost weight during the first week or so, and then gained weight, remaining very close to the control mice, except for the high dose females. The latter's body weights at 12 and 24 months were significantly (Dunnett's multiple comparisons procedures) below those of the controls. There was no terminal body weight for the high dose males, since the last high dose male died just before the study termination. This was not a dose-related phenomenon, as after 24 months there was only one surviving male control, and four and two survivors in, respectively, the low and the middle dose groups.

Fifty per cent of the high dose males had died by ~16.5 months and 50% of the control males had died by ~ 17.5 months. These two events did not occur in the female mice until ~20.5 and 22 months respectively. Ultimate survival of the females also was greater than that of the males as shown below.

SURVIVAL IN FEMALE MICE				
	PER CENT TNG IN DIET			
	0.0	0.01	0.1	1.0
rs at 24 Months	18	16	12	10
rs at 24 + 1 Months[1]	2/4	3/4	2/4	2/4

1. All but four mice in each group were sacrificed at 24 months.

There appears to be a dose-response relationship in the female mouse survival, but the investigators did not report that they had evaluated this aspect statistically. This survival incidence probably is just coincidence (Comment of author of Robust Summary). The males had one control, four low-dose, two middle dose, and (as previously mentioned) no high dose survivors after 24 months. Except for the control group, there appears to be a dose-response relationship here also, but the numbers are so small that this also may be coincidence; particularly in view of only one survivor in the Control group (Comment of author of Robust Summary).

Two middle dose females had severely depressed erythrocyte counts at 24 months, and one of the two had extreme reticulocytosis. However, the latter had no Heinz bodies and the former had only a 0.4% concentration of Heinz bodies in her erythrocytes. This is in contrast to concurrent incidences as high as 2.88 % in the erythocytes of high dose females having depressed erythrocyte counts. The investigators concluded that the anemia in the middle dose females was a typical geriatric anemia, rather than agent-induced.

This conclusion was supported by the clinical picture of one of the two control female mice allowed to recover for a month after 24 months of dosing with TNG. The affected one had a 40% reduction in erythrocyte count, a 24.2% incidence of reticulocytes and no Heinz bodies.

There were isolated instances of significantly (Dunnett's multiple comparisons procedures) elevated or decreased organ or body weights in the high dose mice:

1. Females - Ratio of heart wt. / body wt. significantly increased (24 months).
2. Males - Kidney wts. significantly reduced (12 month + 1 month recovery).
   Ratio of spleen wt./brain weight significantly increased (12 months).

The middle dose male group had significantly (Dunnett's multiple comparisons procedures) elevated hemoglobin and methemoglobin values at twelve months. However, the high dose group's hemoglobin concentration was lower and it's methem-oglobin was the same as that of the middle dose group. The middle dose group's hemoglobin and methemoglobin values were normal at the 12 + 1 recovery measurement and in all future analyses. The investigators did not consider this a TNG-related event. The mean corpuscular hemoglobin level (picograms) of the middle dose group was significantly elevated at the 12 month recovery measurement, but again this was an isolated event and there was no dose-response relationship with the high dose group's level. Again, the investigators did not consider this a TNG-related event.

CONCLUSIONS

Whether the "No Effect" level is the middle (114.6 ± 4.6 mg TNG / kg / day in males and 96.4 ± 3.3 mg TNG / kg / day in females) or the low dose (11.10 ± 0.40 mg TNG / kg / day in males and 9.72 ± 0.29 mg NG / kg / day in females), or an even lower dose, probably is moot for two reasons (Opinion of the author of this Robust Summary):

1. The principal effect seen at the middle dose in males and females was a low incidence of pigment deposits in the liver from males and females in the "Unscheduled Deaths" category. This effect would have disappeared at dose levels equal to the NOAEL of TNG in rats: 3.04 ± 0.16 mg TNG / kg / day in males and 3.99 ± 0.18 mg TNG / kg / day in females.
2. The toxicologically determining factor almost certainly will be the hepatocellular carcinomas seen in the male rats at 31.5 ± 1.6 mg TNG / kg / day and in the female rats at 38.1 ± 1.6 mg TNG / kg / day.

DATA QUALITY

The data themselves are **GOOD** data. The study is well and thoroughly designed overall. The study suffers from the lack of male survivors at 24 months. The study suffers statistically because of the small number of subjects at the interim sacrifices and recovery studies. The latter deficiency can be overcome to some extent by use of the quality data from the unscheduled deaths. The rat study overcame these deficiencies by having multiple interim sacrifices, with these same numbers of animals at each sacrifice. Thus the rat study overcame, in the aggregate, the deficiency of each sacrifice (Opinion of the author of this Robust Summary). This is a **KEY STUDY**.

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MAMMALIAN TOXICITY ELEMENTS
17) TOXICITY TO REPRODUCTION
(DIETARY ADMINISTRATION)

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These all are cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for NG.

PURITY

Pure TNG was used to prepare a stock diet concentrate from feed dried to 0.1 % moisture. The concentrate was analyzed by GC (flame ionization detector). No peaks other than TNG were seen, with a detection limit (*vs*. TNG = 100%) of 1 % for other components. It also was analyzed for TNG *per se* by the method of Wells (1970). (This information was used to guide the weekly preparation of the diets.) In addition, a sample of the diet was extracted with chloroform and an aliquot of the extract was evaporated to yield a residue whose infrared spectrum (between salt plates) was determined. It was identical to that reported for TNG (Hayden, *et al*., (1972).

The diet concentrate was stored in plastic bags which were then stored in five gallon glass jars sealed by wooden plugs covered with aluminum foil. The plugs were held in place by springs. The jars were stored in an underground magazine. Test levels of diet were prepared fresh weekly by dilution of the concentrate with unheated feed and were analyzed after preparation. Control analyses were carried out over eight-day periods to measure evaporation of NG from the diet under cage conditions during the week. GC also was used for these analyses, but with a ^{63}Ni detector. This information was used to calculate actual dosage received by the rats. Feeders were topped off with fresh diet on the fourth day of each week, and diet in each cage feeder was replaced totally every seven days. The feed for the control rats contained 10% (w/w) of the diet dried to 0.1 % water content.

METHOD

GUIDELINE FOLLOWED: U.S.F.D.A Guidelines (1966). F_0 group consisted of 10 males and 20 females cohabited for 14 days. F_{1a} group was discarded and the F_0 group remated. Twenty to 24 apparently normal offspring (F_{1b}) of each sex were randomly selected in approx. equal numbers from each group and mated 1 / 1 with rats from the same group. The same procedure was followed with the F_2 group. The F_{3b} group was sacrificed after weaning and the overt physical and reproductive parameters called for in the FDA Guideline were evaluated. The results are shown in the tables on the pages 4 and 5. As usual, rats in each cage were examined daily.

TEST TYPE: Reproduction, Three Generation.

GLP: Unlikely. Predated GLP.

YEAR PERFORMED: 1976.

SPECIES: Albino rat.

STRAIN: Charles River CD.

ROUTE of ADMINISTRATION**: Dietary.**

DOSES**: 0.0, 0.01, 0.1, and 1.0 % (w/w) in the diet. Corresponding average TNG intakes for six months pretreatment (six months for males and five months for females) were, respectively, 0.00(M & F); 3.60 ± 0.28(M), 5.00 ± 0.17(F); 39.0 ± 1.8(M), 46.0 ± 0.9(F); and 408 ± 18(M), 452 ± 9(F) mg TNG / kg of rat / day. Females were dosed during pregnancy and between matings. Males were dosed until successful delivery of each "b" generation.**

DEVIATIONS FROM PROTOCOL: The F_{1b} dams receiving the 1.0% TNG dose in their diets produced only three F_{2a} litters. Therefore, 14 pairs of F_{2a} rats (instead of F_{2b} rats) were used in an attempt to produce the F_3 litters. In two matings they produced only one F_{3a} litter. The F_{2a} females were then mated to contemporary control males and 13 of the 14 F_{2a} females became pregnant and successfully carried to day 20. During the F_{1b} gestation period, food intake of the high dose test and control dams was measured.

RESULTS

The results of the study evaluated per the protocol used are summarized in the tables on the pages 4 and 5. The conclusion below that the F_{2a} males were impotent was strengthened by the observations that 1) their testes were ~25 % of the normal size, 2) they had produced a large number of vaginal plugs without sperm, and 3) microscopic examination of slides prepared from their testes revealed severe aspermatogenesis and mild-moderate increases in the amounts of interstitial tissue in their testes.

All litter parameters except male / female ratio were reduced in the high dose F_{1a} litters. Most parameters also were somewhat reduced in the high dose F_{1b} and F_{2a} litters.

The food intake of the F_{1b} dams was ~65 % that of the corresponding control dams. Their gestational product (litter size x litter weight) was ~62 % that of those control dams.

CONCLUSIONS

The high dose (1.0 % in the diet; 6-month average daily male intake = 408 mg ± 18 mg TNG / kg / day) caused severe aspermatogenesis with resulting severe infertility in the F_{2a} generation of males. The high dose in the females (452 ± 9 mg TNG / kg

/ day caused a slight reduction in the parameters measured in the high dose F_{1b} litter. Measurement of the dams' food intake during the F_{1b} gestation period indicated a 35 % reduction of food intake *vs*. the control rats. This probably was responsible for the 38 % reduction in the individual total litter weights observed in the high dose F_{1b} litter.

Negative dominant lethal mutagenic and teratogenic studies (Robust Summaries included in this set), suggested strongly that these reduced litter parameters were not due to mutagenic or teratogenic effects as measured by those studies in intact rodents.

Male daily dietary intake of an average 408 ± 18 mg TNG / kg / day was an effect level in male rats as measured by aspermatogenesis in the F_2 generation of males. Female dietary intake of 452 ± 9 mg TNG / kg / day was an effect level in females as measured by litter size and birth weights. Dietary intakes of 39 ± 1.8 and 46 ± 0.9 mg TNG / kg / day, respectively, were no-effect levels for males and females.

DATA QUALITY

Very Good, but lacking some of the measurements called for in the current OECD Guideline. **KEY STUDY.**

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AGE, WEIGHT, AND FERTILITY OF THREE GENERATIONS OF RATS GIVEN DIETARY TNG

TNG (% in feed)	Genera-tion	Female age at First Mating (Months)	Mating Ratio	Pregnancy Ratio	MALES		FEMALES		Duration of Gestation (Days)
					Fertile / Mated	Wt. at 1st Mating (g)	Fertile/ Mated	Wt. at 1st Mating (g)	
0	F_0	5	41 / 48[1]	37 / 41[2]	10 / 10	608 ± 15[3]	23 / 24	305 ± 4	22.6
	F_1	5	35 / 40	24 / 35	17 / 20	590 ± 12	15 / 20	308 ± 5	22.1
	F_2	4	35 / 40	31 / 35	19 / 20	538 ± 8	19 / 20	293 ± 6	22.1
0.01	F_0	5	39 / 47	33 / 39	9 / 10	649 ± 21	19 / 24[4]	328 ± 5[5]	23.0
	F_1	5	36/ 40[4]	32 / 36[4]	19 / 20	669 ± 10[5]	19 / 20[4]	328 ± 8	22.4
	F_2	4	33/ 39	28 / 33	18 / 20	555 ± 10	17 / 20	321 ± 9[5]	22.2
0.1	F_0	5	43 /46	37 / 43	10 / 10	604 ± 22	24 / 24	309 ± 4	22.7
	F_1	5	36 / 40	32 / 36[4]	20 / 20	643 ± 10	20 / 20[4]	308 ± 5	22.3
	F_2	4	38 / 40	37 / 38	19 / 20	520 ± 8	20 / 20	288 ± 6	22.0
1.0	F_0	5	41 / 47	36 / 41	9 / 9	433 ± 18[5]	23 / 24	247 ± 4[5]	22.9
	F_1	5	35 / 40	8 / 35[4]	6 / 20[4]	337 ± 9[5]	8 / 20[4]	217 ± 5[5]	22.4
	F_2[6]	4	22 / 28	1 / 22[4]	1 / 14[4]	336 ± 11[5]	1 / 14[4]	225 ± 6[5]	23.0

1. Number of copulations detected by vaginal smear to the number of male-female pairings.
2. Number of confirmed pregnancies to the number of copulations.
3. Mean ± standard error.
4. Significantly different from the ratio for the respective control generation (Fisher's exact probability test).
5. Significantly different from the mean value of the respective control generation (Dunnett's multiple comparison procedure).
6. Derived from first litters of the F_1 generation.

REPRODUCTIVE PERFORMANCE OF FEMALE RATS GIVEN TNG IN A THREE-GENERATION DIETARY STUDY									
TNG (% In Feed)	Litter No.	Litter Size	Live-born Index	Birth Weight (gm)	Viability Index	Lactation Index	Weight at Weaning (gm)	M / M+F At Weaning	Feed Intake (gm) During Gestation
0	F_{1a}	14.3 ± 0.7 (17)[1]	96 ± 2	7.6 ± 0.2	99 ± 1	93 ± 6	51 ± 3	83 / 167	
	F_{1b}	14.5 ± 1.0 (13)	98 ± 2	7.8 ± 0.3	99 ± 1	85 ± 6	49 ± 3	58 / 121	627 ± 51
	F_{2a}	13.9 ± 0.5 (13)	100	6.7 ± 0.2	100	98 ± 1	41 ± 2	98 / 178	
	F_{2b}	15.2 ± 0.5 (8)	100	7.1 ± 0.2	99 ± 1	84 ± 10	41 ± 2	32 / 103	
	F_{3a}	13.8 ± 0.8 (15)	100	7.1 ± 0.2	99 ± 1	99 ± 1	43 ± 2	87 / 202	
	F_{3b}	13.1 ± 1.4 (12)	98 ± 2	7.1 ± 0.2	97 ± 4	89 ± 8	36 ± 4	77 / 139	
0.01	F_{1a}	10.8 ± 0.9 (16)	83 ± 8	6.9 ± 0.7	74 ± 11	83 ± 10	47 ± 6	67 / 131	
	F_{1b}	10.1 ± 0.7 (8)	99 ± 1	7.8 ± 0.3	97 ± 2	85 ± 8	42 ± 2	39 / 88	621 ± 27
	F_{2a}	12.9 ± 0.1 (16)	99 ± 1	7.3 ± 2[2]	99 ± 1	93 ± 6	43 ± 4	97 / 198	
	F_{2b}	12.9 ± 1.5 (13)	97 ± 3	7.0 ± 0.2	93 ± 8	88 ± 4	47 ± 3	82 / 142	
	F_{3a}	10.6 ± 0.9 (14)	98 ± 2	7.8 ± 0.4	100 ± 5	99 ± 1	47 ± 3	88 / 179	
	F_{3b}	15.8 ± 0.9 (11)	98 ± 2	6.8 ± 0.1	94 ± 3	90 ± 5	40 ± 2	74 /142	
0.1	F_{1a}	9.9 ± 0.9 (20)	89 ± 5	7.9 ± 0.2	75 ± 10	94 ± 5	53 ± 2	81 / 158	
	F_{1b}	11.1 ± 1.1 (13)	95 ± 4	8.1 ± 0.3	97 ± 2	89 ± 4	48 ± 3	62 / 117	591 ± 20
	F_{2a}	14.7 ± 1.0 (19)	98 ± 1	7.1 ± 0.1	98 ± 1	98 ± 1	46 ± 3	97 / 206	
	F_{2b}	14.1 ± 1.1 (12)	94 ± 3	6.9 ± 0.2	97 ± 1	95 ± 2	42 ± 2	68 / 147	
	F_{3a}	10.4 ± 0.7 (19)	98 ± 1	7.1 ± 0.2	98 ± 1	99 ± 1	42 ± 2	110 / 208	
	F_{3b}	14.8 ± 0.4 (15)	98 ± 1	6.8 ± 0.1	100 ± 1	96 ± 1	39 ± 1	104 / 208	
1.0	F_{1a}	6.9 ± 0.9 (14)[2]	59 ± 11[3]	5.9 ± 0.2[3]	35 ± 11[3]	35 ± 12[3]	10 ± 3[3]	13 / 30	
	F_{1b}	9.7 ± 0.9 (9)	99± 1	5.8 ± 0.2[2]	75 ± 8[3]	67 ± 12	26 ± 4[2]	13 / 37	409 ± 39[2]
	F_{2a}	7.0 ± 1.5 (3)[3]	83 ± 17	5.4 ± 0.2[2]	83 ± 17	84 ± 8	30 ± 7	8 / 14	

1. Mean ± standard error and, in parentheses, the number of litters included in the mean.

2. Significantly different from the mean value of the respective control litters (Tukey's omega procedure).

3. Significantly different from the mean value of the respective control litters (two sample rank test).

MAMMALIAN TOXICITY ELEMENTS

18) DEVELOPMENTAL TOXICITY

RAT (DIETARY) TERATOGENICITY

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for TNG in the National Library of Medicine (NLM) "ChemIDplus" data base. These all are cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

Pure TNG was used to prepare a stock diet concentrate from feed dried to 0.1 % moisture. The concentrate was analyzed by GC (flame ionization detector). No peaks other than TNG were seen, with a detection limit (*vs*. TNG = 100%) of 1 % for other components. It also was analyzed for TNG *per se* by the method of Wells (1970). (This information was used to guide the weekly preparation of the diets.) In addition, a sample of the diet was extracted with chloroform and an aliquot of the extract was evaporated to yield a residue whose infrared spectrum (between salt plates) was determined. It was identical to that reported for TNG (Hayden, *et al*., (1972).

The diet concentrate was stored in plastic bags, which were then stored in five gallon glass jars sealed by wooden plugs covered with aluminum foil. The plugs were held in place by springs. The jars were stored in an underground magazine. Test levels of diet were prepared fresh weekly by dilution of the concentrate with unheated feed and were analyzed after preparation. Control analyses were carried out over eight-day periods to measure evaporation of TNG from the diet under cage conditions during the week. GC also was used for these analyses, but with a ^{63}Ni detector. This information was used to calculate actual dosage received by the rats. Feeders were topped off with fresh diet on the fourth day of each week, and diet in each cage feeder was replaced totally every seven days. The feed for the control rats contained 10% (w/w) of the diet dried to 0.1 % water content.

METHOD

METHOD FOLLOWED: FDA Guidelines (1966). Sexually mature virgin females were mated with experienced young adult male rats. The dams' F_{1C} litter was used for this study. The dams were placed on test or control diets for gestation days 6-15, incl., and were sacrificed (CO_2 inhalation) on gestation day 20.

TEST TYPE: Oral administration in diet.

GLP: Unlikely. Study pre-dated even USFDA GLPs

YEAR: 1976

SPECIES: Albino rat.

STRAIN: Charles River CD.

SEX: Females

ROUTE OF ADMINISTRATION: Diet

DOSE LEVELS: 0.0, 0.01, 0.1, 1.0 % TNG (w/w); probably ~ 60 mg/kg/day at the high dose level (see Genetic Toxicity, Rat Kidney Cells, Subacute and Subchronic Administration

EXPOSURE PERIOD: Twenty-four hours / day for 10 days.

STATISTICAL METHODS: Mean or mean ± standard error.

REMARKS **: See table below for Day 0 weights of females. Females were examined by vaginal lavage in late afternoon and, if signs of proestrus were present, were then placed with the experienced male overnight in the ratio of two females / male. The next morning the females were examined for the presence of sperm or a vaginal plug. During pregnancy the dams were observed daily.**

***Corpora lutea*, as well as numbers and positions of live, dead, and resorbed fetuses were determined at necropsy. Fetuses were then removed from the uterus, weighed, and examined for any external anomalies. Half the fetuses from each litter were then fixed in Bouin's fluid, manually sectioned, and examined for soft tissue /internal organ anomalies by Wilson's method (Wilson, 1965). The remaining fetuses were fixed in 70% ethanol followed by 1% potassium hydroxide and then stained with alizarin red (Staples and Schnell, 1964). This was followed by differential decolorization and examination for skeletal anomalies.**

GENERAL COMMENTS: This test was part of a suite of tests designed and implemented in the mid-seventies to do a complete toxicological evaluation for nitroglycerin and other munitions chemicals, using then-contemporary test standards.

RESULTS

The parameters evaluated, and their values are given in the following table.

REPRODUCTIVE PERFORMANCE OF FEMALE RATS GIVEN **TNG IN FEED AND MATED WITH UNTREATED MALES**				
	PER CENT TNG IN FEED			
	0	**0.01**	**0.1**	**1.0**
Mated[1]	21	21	20	22
Sperm positive[2]	17	11	15	20
Pregnant	15	9	12	19
Maternal weight, Day 0	349 ± 6[3]	343 ± 9	346 ± 8	264 ± 4[4]

Corrected weight change[5]	47 ± 6	63 ± 3	40 ± 7	25 ± 4[4]
Liver weight Relative to corrected weight[6]	15.7 ± 0.5	16.4 ± 0.8	15.7 ± 0.7	18.2 ± 0.8
	4.0 ± 0.1	4.0 ± 0.2	3.8 ± 0.4	6.3 ± 0.2[4]
Implants / dam Viable fetuses (%)[7] Dead fetuses (%)[7] Early resorptions (%)7 Late resorptions (%)[7]	11.6 ± 1.1	13.7 ± 1.4	11.0 ± 1.2	12.8 ± 0.7
	89 ± 4	84 ± 6	98 ± 4	93 ± 2
	0	0	0	0
	10 ± 4	9 ± 3	6 ± 4	3 ± 1
	1.3 ± 0.9	7.6 ± 3.8	0.6 ± 0.6	4.3 ± 1.8
Dams with complete resorptions	0	0	0	0
Live litters Fetuses / dam Males (%)[7] Fetal weight (gm)	15	9	12	19
	10.4 ± 1.2	11.6 ± 1.5	10.2 ± 1.3	11.8 ± 0.7
	45 ± 3	48 ± 6	53 ± 5	46 ± 4
	2.77 ± 0.28	3.20 ± 0.18	3.25 ± 0.09	2.81 ± 0.08
Soft tissue anomalies Daphragmatic hernia (%)[7]				
	0	0	0	3.7 ± 1.7
Skeletal anomalies Hyoid bone Unossified (%)[7] Incompletely ossified (%)[7]				
	2 ± 1	4 ± 3	3 ± 3	29 ± 7[8]
	3 ± 3	6 ± 5	0 ± 0	19 ± 5[8]
Sternabrae unossified	78 ± 8	65 ± 14	71 ± 11	87 ± 5

3. Exposed to females.
4. Sperm found in the vaginal smear.
5. Mean or mean ± standard error (S.E.).
6. Significantly different from control (Dunnett's multiple comparison procedure).
7. Dam body weight [(Day 20 – Day 0) – uterine weight on Day 20].
8. Grams of liver / 100gm corrected body weight (Day 0 weight. + corrected weight change).
9. Mean ± S.E of the per cent of fetuses with the indicated characteristic calculated on a "per litter" basis.
10. Significantly different from control ($p < 0.05$, two-sample rank test).

CONCLUSIONS OF INVESTIGATORS

The weights of the females at termination and their weight changes (excluding the uterus and contents) were significantly less than those of the control group and less than those of the two groups tested at lower doses, as indicated in the summary table above. In addition, their liver weights were significantly increased relative to their corrected body weight and increased relative to those of the two lower dose test groups. Diaphragmatic hernias occurred only in the high dose group and were believed, by the investigators, to be due to the test material. Their incidence was not significant (two-sample rank test), but they occurred in 4 / 19 of the high dose litters from this study. The incidences of unossified and incompletely ossified hyoid bones also were significantly increased compared to the controls and were increased compared to the two lower dose test groups. However, sternabrae, centra, and skull bones were not similarly affected. The investigators believe that these diaphragmatic hernias may have been at least partially responsible for the poor reproductive performance of the group dosed at the1.0 % (w/w) dietary level in the three-generation reproduction test (See that Robust Summary for data).

DATA QUALITY

Very Good. (Comment of author of this Robust Summary.). **KEY STUDY.**

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SIDS_TNG_TeratoRatDietaryFinal

HUMAN HEALTH EFFECTS

SPECIAL REPEATED DOSE TOXICITY

KINETICS OF METHEMOGLOBIN FORMATION & DISAPPEARANCE

METHEMOGLOBINEMIA PROTECTION / ANTIDOTE

FIVE DOSE ORAL STUDY IN DOGS

TEST SUBSTANCE – IDENTITY

- Nitroglycerin (TNG; 1,2,3-propanetriol, 1,2,3-trinitrate; CASRN 55-63-0)

- Synonyms include almost 100 trivial and trade names (CCOHS, 2001).

There are four alternate CASRNs: 8013-32-8, 9010-02-0, 80066-48-4, and 105469-31-6 listed for nitroglycerin (TNG) in the National Library of Medicine (NLM) "ChemIDplus" data base. These all are cross-referenced to 55-63-0 in the NLM and the Canadian Center for Occupational Health & Safety (CCOHS) toxicological data bases (CCOHS, 2001). The numbers 8013-32-8 and 105469-31-6 are not listed in the Chemical Abstracts Service Registry Handbook, Number Section. Number 9010-02-0 is attributed to "SNG" (no further identification) in this Handbook Section. Number 80066-48-4 is attributed in this Handbook Section to 1,2,3-propanetriol, 1,2,3-trinitrate, the systematic name for TNG.

PURITY

Production grade TNG adsorbed on lactose to give a product analyzing for 9.72 ± 0.09% (w/w) NG was used in the capsules with which the dogs were dosed once daily. It was analyzed by GC (flame ionization detector). No peaks other than those from NG were seen, with a detection limit (*vs*. TNG = 100%) of 1 % for other components. It also was analyzed for TNG *per se* by the method of Wells (1970). In addition, a sample of the TNG/lactose test material was extracted with chloroform and an aliquot of the extract was evaporated to yield a residue whose infrared spectrum (between salt plates) was determined. It was identical to that reported for TNG (Hayden, *et al*., (1972).

OBJECTIVES OF STUDY

The study had two objectives: 1) to determine any dose response relationship for the appearance and disappearance of methemoglobin in dog blood following repeated once-daily oral bolus doses of a series of large amounts of TNG and 2) to evaluate any therapeutic / protective effect of methylene blue administered intravenously two hours after an oral bolus dose of 200 mg / kg of TNG. The report contained no rationale for either the size of the test dose of TNG evaluated, or the dose of methylene blue evaluated.

METHOD

METHOD FOLLOWED: Protocol designed by the investigators. See below for outline of protocol. There is no OECD protocol for this type of study.
TEST TYPE: Repeated daily administration of TNG at levels expected to cause methemo-globinemia.
GLP: Probably not. Study pre-dated even USFDA GLP codification.
YEAR: 1975.
SPECIES: Dog; Beagle. Young adults from Hazleton Research Animals, Cumberland, VA.
SEX: Male & female.
ROUTES of ADMINISTRATION: Oral via capsule (TNG), and i.v. (methylene blue).
DURATION: Five days (one dose / day); no recovery period
DOSES: 25, 50, 100, or 200 mg TNG / kg in a bolus dose of one or more capsules administered successively each day. Three milliliters of methylene blue intravenously to 200 mg TNG / kg / day group on Day 3 (See RESULTS table).
MEASUREMENTS: Blood hemoglobin and methemoglobin.
GROUP SIZE: Two males and two females.
CONTROL GROUP: None
STATISTICAL METHOD: Average and range of both sexes combined.

RESULTS

BLOOD LEVELS of METHEMOGLOBIN (% of Total Hemoglobin)[1]								
TEST DAY	TNG Dose (mg/kg/day)	HOURS (Post-dose)						
		1 / 2	1	2	4	8	16	24
1	25	0	0.6	5.7	7.3	0	0	0
	50	0	4.4	12.0	15.7	6.0	0	0
	100	0.60	2.4	12.3	25.6	25.9	4.6	0.7
	200[2]	0	2.9	11.2	28.3	16.6	0	0
2	25	0	0	1.6	2.8	0	0	0
	50	0	0.7	4.3	9.2	3.0	0	0
	100	0.8	2.4	10.7	24.7	20.9	1.5	0
	200	0	0	25.9	50.7	26.8	7.3	0
3	25	0	0	4.1	8.7	1.6	0	0.8
	50	0	0	8.7	13.0	5.8	0	0.8
	100	0	1.6	18.1	42.6	33.1	0.8	0.8
	200	0	0	31.0[3]	16.3	25.6	16.5	3.6
4	25	1.6	0	3.1	5.6	0.9	0	0
	50	0	0	5.5	14.1	4.1	0	0
	100	1.8	0.9	11.5	39.0	30.2	5.1	0
	200[4]	4.0	--	2.1	5.5	3.8	0	0
5	25	0	0	2.9	2.8	1.3	0	0
	50	0	0	7.5	12.0	2.2	0	0

	100	0.7	2.2	22.6	38.5	22.6	3.0	0
	200[4]	0	0	0	0	0	0	0

3. Average of two males and two females combined. Range not given in report.
2. One dog vomited after dosing, but its methemoglobin percentages are included.
3. All dogs in this group treated with methylene blue (3 mg / kg) on this day, two hours after TNG dose.
4. This group received no TNG on days four & five.

CLINICAL SIGNS: None, except cyanosis and inactivity for 2 – 3 hours each day after receiving 100 or 200 mg TNG / kg. The inactivity appeared to be dose-related, but the time-to-appearance and duration of inactivity did not appear to be.

REMARKS: The study suggests two things: 1) mammals can cope, within 16 hours, with methemoglobin levels below 25%, and within 24 hours, with levels as high as 40%, and 2) intravenous methylene blue will retard the formation of methemoglobin caused by TNG, but will not accelerate the removal of methemoglobin.

DATA QUALITY

The data set is good, the author of this Robust Summary believes the numbers are reliable, and that methylene blue probably will not be an effective antidote for human methemoglobinemia caused by TNG. This is a **KEY STUDY**.

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